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A Textbook of Microbiology

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THE MACMILLAN COMPANY
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PART ONE

FUNDAMENTALS OF MICROBIOLOGY

CHAPTER I

THE SCOPE AND CHARACTER OF MICROBIOLOGY

THE FIRST thing to learn in beginning the study of microbiology is the existence of what may be called the *world of microbes.* We must realize that the familiar plants and animals are not the only living forms on the earth, but, on the contrary, there are all about us everywhere great numbers of very tiny living creatures, too small to be seen by the naked eye. We are ordinarily quite unconscious of their presence; if we wish to see them, it is necessary to use a microscope. They are appropriately called *micro-organisms* or *microbes* (from the Greek *micros,* meaning small, and *bios,* meaning life), and may be defined as *living forms of microscopic size.*

Micro-organisms. Conspicuous among the microbes are those belonging to the group called **bacteria.** These are simple microscopic plants. The word *bacteria* comes from a Greek word meaning "a rod." Some, but not all, of the bacteria have the shape of little rods, or sticks. *Bacteria* is a plural word and is used in a collective sense to refer to the whole group. When we wish to speak of a single one of these microscopic organisms, we use the singular, *bacterium.*

The bacteria of simplest structure are called the **true bacteria.** Those of more complex make-up, resembling the common molds in some respects, are spoken of as the **mold-like higher bacteria.** In another group are included certain peculiar, flexible, spiral-shaped microbes, that seem to be more animal-like than plant-like, yet have basic properties in common with the true bacteria. These we call the **spirochetes.**

These various forms of the bacteria are so numerous and so widely distributed, and their activities are of such great practical importance, that they are often given the major share of attention in academic courses dealing with the microbes, and commonly the entire study is called *bacteriology*. We are concerned not only with the bacteria, however, but with *all* the types of microbes. Hence, the title adopted for this book—*microbiology*—is a more accurate name for our study.

Besides the bacteria, there are four other important groups of micro-organisms. These are: (1) the **protozoa,** (2) the **true fungi,** (3) the **rickettsiae,** and (4) the **filtrable viruses.**

The *protozoa* are relatively large and complex, animal-like organisms, the lowest forms of life classed in the animal kingdom. The special study of these forms is the science of *protozoology*.

The *true fungi* include yeasts, molds, and other microscopic plant-like forms of more complex nature than the true bacteria. The intimate study of the fungi is called *mycology*.

The *rickettsiae* are peculiar microbes smaller than most bacteria, but still visible under the microscope. They are so closely adapted to life within the tissues of their human and animal hosts that they cannot be cultivated in the laboratory on artificial, nonliving, food mixtures.

The *filtrable viruses* are the smallest of all the disease-producing agents. They are invisible by ordinary methods of microscopic examination (i.e., they are ultra-microscopic), and like the rickettsiae, they refuse to grow in the laboratory on lifeless materials.

It is helpful to have in mind, at the beginning of our study, the seven groups of micro-organisms we have mentioned: (1) *protozoa*, (2) *true fungi*, (3) *mold-like higher bacteria*, (4) *spirochetes*, (5) *true bacteria*, (6) *rickettsiae*, and (7) *filtrable viruses* (see Fig. 11).

The bacteria and other micro-organisms are often called *germs*. It is permissible to employ this term in a popular sense when referring to *disease-producing* organisms, but it should not be used to include all varieties of microbes, because it implies that they are all harmful, and this is untrue. The fact is that *the great majority of micro-organisms are entirely harmless* and have no connection whatever with disease. Many are very useful to man.

Scope of microbiology. Microbiology is the science which treats of the nature and activities of micro-organisms.

Since the microbes are *living*, it follows that we are studying one branch of the great science of *Biology*, which includes all studies dealing with the nature of living matter and the character and functions of living things. The relation of microbiology to other biological sciences is indicated in the outline on page 4.

So vast is now the accumulated knowledge about the microbes and their activities that no one microbiologist can claim to be intimately familiar with all aspects of the subject, nor could the whole of microbiology be taught in a single course, or described in a single textbook. As the outline shows, the science of bacteriology alone may be divided into several branches, or subsciences, each dealing with an important phase of bacterial activity. Similarly, protozoology and mycology might be subdivided into subsidiary branches of study.

In this book we are concerned principally with the *medical* aspects of microbiology. Our subject matter includes not merely a description of the disease germs themselves, but also a study of the way in which these organisms cause disease, how we "catch" disease, how the germs injure our bodies, and how they pass from one person to another. Also, the defense the body puts up against the germs and the factors which explain resistance to disease are studied, and so important and complex is this special phase of the subject that it is given a name to itself—*immunology*. Finally, the study of medical microbiology and immunology furnishes the basic knowledge on which depends the diagnosis and prevention of germ diseases. It, therefore, forms the very foundation for the intelligent promotion of both the individual and the public health.

Practical nature of microbiology. It will be evident from this brief outline that microbiology is a very practical subject. It includes a great deal more than a mere description of strange microscopic organisms. Of course, some study of the form, structure, mode of life, etc., of micro-organisms must be included in order to understand them, but the subject is concerned principally with the things microbes *do*, and with the practical effects of their activities on human affairs. Many of the most familiar natural changes going on about us continually, such as the souring of milk, the spoiling of food, and the decomposition of the dead bodies of plants and animals, are brought about by bacteria or fungi. These microbes have a direct influence upon the fertility of the soil. They play an important part in various industrial processes, such as the manufacture of leather. Thus the dairyman, the agriculturalist, and the manufacturer are vitally interested in certain kinds of microbes and for very practical reasons.

Relation of Microbiology to Other Biological Sciences

- *Biology* (Study of living things)
  - *Zoology* (Study of animal life)
  - *Microbiology* (Study of microscopic forms of life)
    - *Protozoology* (Study of protozoa,—microscopic animal-like forms)
    - *Bacteriology* (Study of bacteria and related organisms, including the spirochetes and rickettsiae, and the filtrable viruses)
      - *General Bacteriology* (Study of fundamental properties of bacteria)
      - *Agricultural Bacteriology* (Study of bacteria in relation to soil fertility and plant diseases)
      - *Food and Dairy Bacteriology* (Study of bacteria in relation to the preparation and preservation of food)
      - *Industrial Bacteriology* (Study of bacteria in relation to industrial processes)
      - *Medical Bacteriology* (Study of bacteria in relation to disease in animals and human beings). Includes study of:
        - (1) Pathogenic bacteria
        - (2) General factors influencing the start and course of germ diseases
        - (3) Resistance (or immunity) to disease (*Immunology*)
        - (4) Application of bacteriology and immunology to diagnosis, prevention, and specific treatment of disease—the basis for *public health work*
    - *Mycology* (Study of microscopic plants other than bacteria—yeasts, molds, and other fungi)
  - *Botany* (Study of plant life)

For the nurse and doctor, some knowledge or microbiology is essential because so many diseases are caused by germs. The nurse finds in microbiology the explanation of the rules of nursing technique which are taught in the hospital. Many procedures which form a part of routine nursing work are designed to destroy, exclude, or avoid germs, and only by understanding the nature of microbes can such techniques be carried out intelligently. When the student has acquired, through her study of microbiology, an acute consciousness of the almost universal presence of living microbes everywhere, she can realize that many apparently unnecessary details of nursing methods are in reality of the highest importance. They are designed to avoid or destroy invisible (and possibly dangerous) microbes, and are necessary for the protection of the nurse herself as well as of the patient.

When the nurse is called upon to care for a patient with a germ disease, it is helpful to her to know something of the nature of the organisms which are responsible for the illness. And since most germ diseases are *communicable*, i.e., are capable of being transmitted more or less readily to other persons, the nurse has the grave responsibility of preventing their spread. She must know how the germs enter the body, and particularly how they leave the body and pass from person to person, and how to destroy them in the discharges from the patient. Otherwise she may carry the disease to other patients or contract it herself.

Nurses are responsible for the technique by which germs are kept out of wounds during operations and in the dressing room. Obviously this exceedingly important part of nursing work could not be carried out safely by a nurse who does not have a clear understanding of how to avoid and destroy micro-organisms.

Frequently the nurse is asked to help in collecting specimens of pus, sputum, etc., or to make smears or cultures from the patient, for purposes of laboratory diagnosis. The proper methods may be learned in microbiology.

The modern methods of preventing and treating germ diseases with vaccines and serums, the value of personal hygiene and sanitation in preventing disease, and other practical information may be obtained in the study of microbiology. This basic knowledge will enable the nurse to teach others how to avoid illness, and in this way she may make a very important contribution to the public health.

REVIEW QUESTIONS—CHAPTER I

1. Define *micro-organism*, *microbe*, *bacteria*, *bacterium*, *germ*.
2. Define *microbiology*. Why is this a better title for our study than *bacteriology?*
3. Name and characterize briefly the seven principal groups of micro-organisms.
4. Define *biology*, *protozoology*, *mycology*.
5. Name five branches of bacteriology. Could protozoology and mycology be divided similarly into subsciences?
6. State briefly the nature of the subjects studied in general, agricultural, food and dairy, and industrial bacteriology.
7. What are the four topics included in medical bacteriology? Define *immunology*.
8. Explain why microbiology is a practical subject.
9. Give some reasons why a knowledge of microbiology is useful to a nurse.

CHAPTER II

HISTORY OF MICROBIOLOGY

MICROBIOLOGY had its beginnings only about sixty years ago. It would not be accurate to say that it was established at any particular date, but the year 1880 may be remembered as the time when the truly modern science was firmly founded. This means that microbiology is a field of comparatively recent development. It dates back no further than our grandfather's day. Many persons still living can remember when germs were little known and their true importance hardly suspected.

A great deal is still unknown. Our information about microbes is far from complete. But additions to our knowledge are being made continually, as the result of investigations going on all over the world. Especially rapid is the advance now being made in our information about filtrable viruses and rickettsiae—organisms that were little understood just a few years ago. Microbiology and Immunology are developing rapidly and the student must expect to learn frequently of new discoveries and new ideas concerning germs and germ diseases.

Periods of development. Microbiology may be said to have had three periods of development: (1) the period prior to 1865, during which there was a slow accumulation of some facts about bacteria and other microbes and a great deal of more or less correct speculation about them, (2) the period between 1865 and 1882, when the foundations of the new science were securely laid, especially through the pioneer work of the French scientist Louis Pasteur, the German physician Robert Koch, and the English surgeon Joseph Lister, and (3) the modern period from 1882 to the present day, during which there has been a very rapid development of information about microbes and a truly revolutionary application of this knowledge to human affairs, especially in medical and public health work.

THE BEGINNINGS OF MICROBIOLOGY

KNOWLEDGE OF MICRO-ORGANISMS BEFORE 1865

Discovery of microbes. Antony van Leeuwenhoek (1632–1723). The existence of bacteria and other micro-organisms was discovered almost 200 years before anything approaching a science of microbiology was evolved. This discovery was made by Antony van Leeuwenhoek (pronounced "Lay-wen-ho͞ok"), a most remarkable man and a unique figure in the history of science. (See Frontispiece).

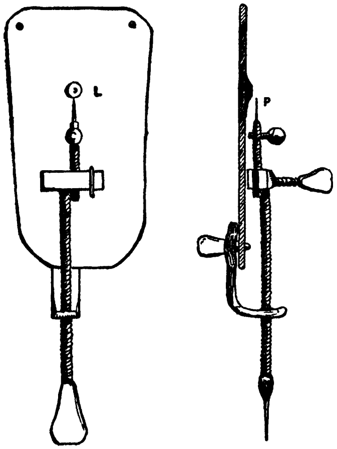

Fig. 1. Front and side view of one of Leeuwenhoek's microscopes. The single magnifying lens was fastened between two thin metal plates at L, and the object to be examined was mounted upon the adjustable pin at P. The object was viewed through the lens by holding the microscope very close to the eye. (Drawn from a photograph.)

Leeuwenhoek, a citizen of Delft, Holland, was not a man of great learning, but he was very ingenious. He became expert in the grinding of simple magnifying lenses. He made these lenses of very small bits of glass, polished them very carefully, and mounted each separately between two copper, silver or gold plates, to which he fastened an adjustable holder for the object to be examined (Fig. 1). He constructed many of these "microscopes," each containing a single lens ground by himself. The best of his lenses magnified about 200 times and they were better than any of the magnifying devices which had been constructed up to his time. With these truly remarkable instruments, he diligently and patiently examined a great variety of natural objects, and described them in a series of letters to the Royal Society of London.

In 1674 he first saw "animalcules," which we now recognize as protozoa and bacteria, in rain-water and in pepper infusions (he was trying to find out what made the pepper hot). Later he found them in the human mouth and in excreta. In his letter of

September 17, 1683 he supplements his quaint descriptions of the "animalcules" in saliva with drawings which depict clearly the same types of bacteria we know to-day.

Early studies which helped to develop microbiology. By 1700 the existence of micro-organisms was probably widely known, but the crude microscopes of that day did not permit much to be learned about them. There were no satisfactory methods for cultivating microbes artificially, or for separating one kind from another. Consequently, for nearly two hundred years following Leeuwenhoek's time definite knowledge about micro-organisms developed very slowly indeed until the work of Pasteur and his immediate followers, beginning about 1860, led directly to the founding of the modern science. In the meantime, however, there were many who studied microbes and these early studies helped to lay the foundation for modern microbiology.

There were four questions which interested the early workers. These were: (1) the classification of micro-organisms and their relation to the larger and more familiar forms of life, (2) the origin of microbes and the truth or falsity of the theory of the spontaneous generation of living things, (3) the relation of micro-organisms to fermentation and putrefaction and (4) the relation of microbes to disease. Important steps in the development of knowledge of these topics up to 1865 are described in the following paragraphs.

Early classifications of bacteria. The first important attempt to classify bacteria was made by Ehrenberg in 1836. He distinguished between spherical, rod-shaped and spiral-shaped bacteria, and was the first to use the terms "bacterium," "spirillum" and "spirochaeta" with reference to bacteria. All of these terms are used to-day, though not with quite the same meaning.

Ehrenberg and all other workers of that time thought the bacteria belonged to the animal kingdom, as Leeuwenhoek himself had believed. It was not until about 1850 that several investigators placed them where they properly belong—in the plant kingdom. About this time Naegeli pointed out that bacteria are colorless plants and are clearly different from green plants. He gave the scientific name of *Schizomycetes*, to the class of micro-organisms we commonly call bacteria. This term means "fission fungi" and is appropriate because bacteria multiply by a process called simple fission—the splitting of one organism into two.

The question of spontaneous generation and the origin of microbes. One important question about bacteria and the other microbes was debated for more than a century. The question was, *What is the origin of micro-organisms? Are they generated spontaneously from the materials on which they are found to be growing, or do they develop, like other kinds of living things, from preexisting organisms of the same kind?* This question had to be answered before a science of bacteriology could be developed.

The debate centered about the origin of the bacteria which appeared in decomposing liquids, such as meat or vegetable infusions. The early investigators noticed that when a perfectly clear infusion was allowed to stand for a time in a warm place it became cloudy, and when the cloudy fluid was examined with the microscope, it was found to be teeming with living micro-organisms. Nearly everyone believed that the microbes developed spontaneously from the liquid itself, that is, the living micro-organisms were thought to be generated from the non-living infusion. This belief was a natural one for that day, because the idea of the *spontaneous generation of life* was a very old one, and with respect to microscopic forms of life, at least, it had never been convincingly disproved. Aristotle and others of the ancient Greek writers taught that living forms develop from non-living matter, and the belief was general throughout the Middle Ages. In the sixteenth century the statement that mice could be created by placing some old linen and corn in a cupboard was believed in all seriousness. Later the absurdity of such an idea became apparent and by the late seventeenth century the better informed persons had ceased to believe that the larger and more familiar plants and animals could spring into being from lifeless matter. But when micro-organisms were discovered, there were many who were ready to believe that these tiny living things must be spontaneously generated.

Experimental studies on spontaneous generation. In 1749 Needham described some experiments which were intended to prove the spontaneous generation of bacteria. He placed vegetable infusions in flasks which he sealed. He then heated the

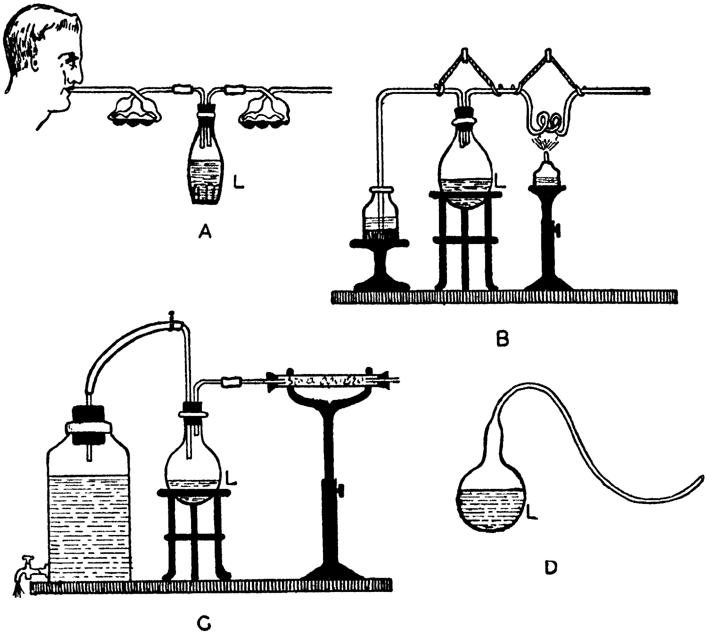

FIG. 2. Illustrating some of the principal experiments which helped to overthrow the belief in "spontaneous generation" of life. The first step in each experiment was to boil the infusions (L) very thoroughly, so as to destroy all living microbes in them. Air was then admitted to the flasks in order to satisfy the believers in spontaneous generation, who insisted that oxygen must be present for life to originate. But the air admitted to the infusions was first freed of living microbes in some way. Under these conditions the infusions remained perfectly clear; no micro-organisms appeared in them, showing that living things could not generate spontaneously from the lifeless liquid.

A, Schulze's experiment. The set of bulbs next to the face contained alkali and the other set concentrated acid. Air was drawn in through the acid and thus freed of microbes before it reached the liquid. B, Schwann's experiment. In this case air could get into the flask only by passing through the coiled glass tube kept hot by the flame. C, Schroeder and Dusch's experiment. The aspirating bottle drew air into the flask through the tube containing cotton at the right. The cotton filtered out the microbes in the air, just as the cotton plugs now used in bacteriological culture tubes protect the culture from air contamination. D, one of Pasteur's U-neck flasks. Although this flask was freely open to the air the infusion remained sterile so long as the microbe-bearing dust which collected in the U did not reach the liquid.

infusions by immersing the flasks in boiling water for a short time. Despite this treatment the fluid in most of his flasks became cloudy and filled with micro-organisms in a few days. Needham thought he had killed all microbes originally in the infusions by the heating, and therefore believed that the growth which appeared in the fluid later must have been spontaneously generated.

But Spallanzani, in 1769, showed the error of this conclusion. He repeated Needham's experiments, but boiled his infusions for a longer period of time than Needham had done and sealed the flasks in the flame. We now realize that, by the prolonged and vigorous boiling of his infusions, Spallanzani killed some resistant forms of microbes which had survived the relatively slight exposure to heat given them by Needham. These boiled infusions remained clear and contained no living organisms so long as the flasks were completely sealed from the air. Spallanzani, therefore, really proved that spontaneous generation of microbes does not occur.

Appert, in 1810, demonstrated that if food were placed in proper containers and subjected to heat, and if then the container were hermetically sealed, the food would remain free from living micro-organisms and would keep without spoiling. The success of this process of canning food was actually a proof that living things come only from living ancestors.

However, the believers in spontaneous generation were not convinced, and thought that Spallanzani and Appert had destroyed the necessary conditions for the generation of living microbes by excluding the oxygen of the air from their containers. This objection was answered by Schulze, in 1836, who showed that a boiled fluid, such as Spallanzani used, would not contain living bacteria even if air were admitted to the flask, *providing the air was first freed of microbes* by passing it through strong sulphuric acid (Fig. 2). Schwann, in 1837, had the same results when he passed the air through very hot tubes before admitting it to his flasks. In 1854, Schroeder and von Dusch demonstrated that boiled infusions would remain clear and free of micro-organisms if the mouth of the flasks were stoppered with a plug of cotton. Unchanged air or other gases could pass freely through the cotton plug and, therefore, the fact that micro-organisms were not generated from the boiled infusions could not be due to the lack of air.

Spontaneous generation disproved by Pasteur and by Tyndall. The question of spontaneous generation was finally settled by the experiments conducted by Pasteur during the years 1860–1865, supplemented, a few years later, by those of Tyndall. Pasteur showed that living microbes are carried about in the air by particles of dust and that a cotton plug serves as a filter, straining out these dust particles. Cotton plugs are now universally used in our tubes and flasks for this very purpose. Pasteur proved by many ingenious experiments that an infusion which has been properly heated, so as to free it entirely of living microbes, will remain without life even though open to the air, so long as dust is not permitted to enter. To contain his boiled infusions, he constructed flasks with a long neck bent into the shape of a "U" (Fig. 2: D). The end of the U-tube was left open, and dust could collect in the open arm of the tube; but so long as the flask remained upright and none of the dust was allowed to reach the liquid, no growth of micro-organisms occurred.

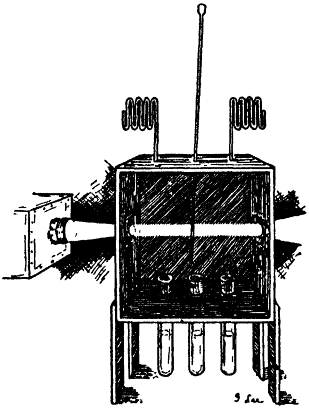

FIG. 3. Tyndall's dust-free chamber. The front side of the box, which has been removed to show the construction, contained a window through which the beam of light could be observed. The inner walls of the chamber were made sticky by coating them with glycerin, and the box was allowed to stand until the bright ray of light crossing the dark interior revealed no motes, that is, until all floating dust particles had settled out. A vegetable infusion, or similar clear fluid, was then introduced through the pipette into the open test tubes, and sterilized by heating the tubes in an oil bath. The convoluted tubing passing through the roof of the box permitted the entrance of air from without, but excluded dust. In these circumstances the fluid in the test tubes remained sterile (free of all microbes) indefinitely.

It was, therefore, established beyond doubt that micro-organisms do *not* generate spontaneously, but *always originate*—as do all other kinds of living things—*by reproduction from parent beings like themselves.* It is one of the fundamental axioms of biology that *living things originate only from pre-existing living things,* and even the tiniest organisms are no exception to this rule.

The truth of this was demonstrated again, a few years later, by the work of John Tyndall (1820–93), the English physicist, whose studies provided the final evidence that served to refute completely the ancient doctrine of spontaneous generation. Tyndall proved by a curious, but very accurate method, that micro-organisms are carried on dust, and that this dust is the source of contamination of fluids exposed to the air. He showed that previously heated fluids (free of all microbes) may be kept in open vessels in a small chamber for long periods without showing any growth of microbes in them, *provided the air of the chamber is dustless,* i.e., free of all floating particles. This he determined by passing a strong beam of light through the sides of the chamber (Fig. 3), and when no motes were thus made visible in the air, he found that fluids could be freely exposed without becoming contaminated.

Studies of fermentation. The souring of milk, the rising of bread dough, the making of alcoholic drinks from sweet fruit juices, and other kinds of fermentation have been known and utilized by peoples all over the world since the very earliest times, but it was not until after the discovery of microbes that the real nature of fermentation could be worked out.

Discovery of yeasts. The most familiar fermentations are brought about by the action of *yeasts,* which are simple microbes belonging to the fungi. Yeasts were seen in fermenting materials long before their significance was understood. It was not until 1837 that Latour and Schwann independently showed that yeasts are living organisms and that they cause the formation of alcohol by the fermentation (decomposition) of sugars as they grow in the sugar solution. Opposition to this view of fermentation was very strong, however, and it remained for Pasteur to furnish convincing demonstration of the relation of micro-organisms to fermentation.

Pasteur's work on fermentation. When Pasteur began his studies, the prevailing opinion regarding fermentation and putrefaction of organic matter was that the changes that go on in the decomposing material were due primarily to the action of the oxygen of the air, and the process was thought to be purely a chemical one. Pasteur was at this time a professor at the University of Lille, France, a town where the making of beers and wines—both products of alcoholic fermentation—was a business of much importance. He studied alcoholic fermentations thoroughly and showed that the changes which occurred in the fermenting matter, with the resulting formation of alcohol, were not simple chemical processes, but were, in fact, brought about by the action of living yeasts. He also demonstrated that other kinds of fermentation, which result in the formation of various other chemical products, are produced by bacteria or by other microbes, and that *each kind of microbe will bring about a characteristic type of fermentation.* His studies made it clear that the *decomposition of lifeless matter of all sorts is caused by the growth of micro-organisms.*

Pasteurization. In connection with his studies of the making of wines and beers, Pasteur found that the so-called "diseases" of wine, which brought much loss to the makers, were caused by undesirable fermentations due to contamination of the original sweet juices with certain kinds of micro-organisms. He further made the important discovery that these undesirable fermentations could be prevented by heating the wine to 60°–65° C. (140°–150° F.). This heating was sufficient to kill the contaminating organisms and yet did not injure the quality of the wine. This process of heating has since been called *pasteurization* and is of immense practical value as now used for destroying any disease germs that may be present in milk.

Early speculations and observations concerning the relation of bacteria to disease. In the earliest medical literature there are vague expressions of the idea that invisible living creatures might be responsible for disease, and for the spread of illness from one person to another. Such references are found, for example, in the writings of Aristotle (384 B.C.) and of a great Arabian physician Rhazes (860–932 A.D.).

In 1546, Fracastoro (1484–1553), a celebrated physician of Verona, published a treatise in which he clearly expressed the belief that invisible living organisms are able to cause disease, and transmit illness by direct or indirect contact from one person to another. A few other writers of the seventeenth century also suggested that minute living organisms might cause disease.

The discoveries of Leeuwenhoek (1674) demonstrated beyond question that micro-organisms really do exist, and ideas concerning their relation to disease gradually became more and more definite as knowledge about them slowly accumulated. The notion that bacteria or other microbes are the real causes of disease—the so-called germ theory of disease—was held by many, though denied by others. Among those who expressed this theory most clearly and forcibly was Anton von Plenciz (1705–1786), a prominent physician of Vienna. He published in 1762 a work in which he presented strong arguments for the idea of living agents as the cause of communicable diseases, and stated that a specific germ probably exists for each different disease.

During the first half of the nineteenth century, many investigators reported the occurrence of micro-organisms of one kind or another in various disease conditions. But proof that these microbes were the actual causes of the diseases in which they were found was generally lacking. It must be remembered that microscopes were still imperfect and successful methods of studying microbes,—especially the tiny bacteria,—had not yet been worked out.

Jacob Henle and "Koch's postulates." In 1840, Jacob Henle (1809–1885), a German scientist, and an immediate predecessor of Pasteur and Koch, expressed the germ theory of disease almost exactly as we now conceive it and also laid down certain necessary procedures which would have to be carried out in order to prove this theory. His argument is generally known today in the form in which it was later stated (1884) by Robert Koch, and these principles have become known as "Koch's laws" or "Koch's postulates."

These postulates declare that, before any organism can be accepted as the cause of a particular disease, all of the following steps must be carried out: (1) the germ must be found in every case of the disease and not in healthy individuals, (2) it must be isolated from the patient and grown in the laboratory apart from all other organisms, (3) it must be inoculated by itself into healthy susceptible animals and must then reproduce the disease, and (4) the same organism must be found again in these inoculated animals and recovered in laboratory cultures.

No one was able to carry out these procedures in 1840 and Henle's admirable ideas had no proof behind them. It was the immortal work of Pasteur, Lister and Koch that finally demonstrated the truth of the germ theory.

REVIEW QUESTIONS—CHAPTER II

1. About what year was microbiology established as an independent science?
2. What are the three periods of time into which the historical development of microbiology is naturally divided?
3. When and by whom were micro-organisms first seen?
4. Describe the microscope made by Leeuwenhoek.
5. What four questions about micro-organisms were studied by early investigators?
6. Who worked out the first important classification of bacteria, and when?
7. When were bacteria recognized as plants rather than animals? What is the scientific name for bacteria and what is the meaning of the term?
8. What is meant by the theory of spontaneous generation? How did Needham try to prove this theory? What was wrong with his experiments?
9. Explain how the experiments of Spallanzani and Appert proved that spontaneous generation does not occur. What was the objection to these experiments on the part of the believers in spontaneous generation?
10. What did Schulze, Schwann, and Schroeder and von Dusch contribute toward settling the question?
11. How and when did Pasteur succeed in definitely disproving the theory of spontaneous generation? How did Tyndall confirm the conclusions of Pasteur?
12. What is meant by alcoholic fermentation? Who discovered yeasts and first showed their relation to fermentation? When?
13. What were the conclusions of Pasteur as to the cause of fermentation and putrefaction? What is the process called pasteurization?
14. Give examples, with dates, of men whose speculations concerning the relation of micro-organisms to disease were close to the truth. Why was it impossible to prove the germ theory at this time?
15. What are "Koch's laws" or "postulates"?

CHAPTER III

HISTORY OF MICROBIOLOGY (Continued)

THE FOUNDING OF BACTERIOLOGY AND IMMUNOLOGY
1865–1882

IN LESS than twenty years following 1865, more progress was made toward a true understanding of micro-organisms and their relation to disease than had been accomplished in all of the preceding time. The most important advances were made as a result of the work of the English surgeon Joseph Lister, the French scientist Louis Pasteur, who was truly the founder of modern bacteriology and immunology, and the German physician Robert Koch, who became the first master of the new science. The perfection of the modern compound microscope also contributed greatly to this rapid development.

Joseph Lister (1827–1912) and antiseptic surgery. Joseph Lister, born in England, April 5, 1827, the son of an eminent scholar, won his degree in medicine in 1852 (Fig. 4). He became professor of surgery at Glasgow and at Edinburgh, and later at King's College in London.

Lister made many contributions to surgery, but his greatest work was the conquest of wound infection. This work was begun while he was at Glasgow, during the years 1865–1869. His first papers on the subject were published in 1867. As a surgeon, he was stirred, as any other kind-hearted man must have been at that time, by the desire to prevent the severe and often fatal inflammations which so frequently followed operations and accidental wounds of any kind. He saw the similarity between the changes that go on in these inflamed wounds and the processes of putrefaction and fermentation which Pasteur had shown to be due to the growth of micro-organisms, and he reasoned that microbes must be the cause of the inflammation in wounds. He saw that bacteria could be kept out of a wound if he would protect it by a dressing saturated with a substance which would kill microbes. This was the basis of his antiseptic method.

As a germicidal substance, Lister chose carbolic acid, which was well known as a preservative. He began by applying carbolic acid dressings to compound fracture wounds—the wounds made when the broken bones protruded through the skin. Wounds of this type always became badly inflamed and frequently led to the death of the patient. The success of the carbolic acid dressing was immediate and astonishing; the wounds healed without the usual quantities of foul pus, and as readily as if there had been no break in the skin at all. Later Lister applied his method to the treatment of abscesses and to other kinds of accidental wounds. Finally, he developed a routine for the use of carbolic acid at operations, which became known as

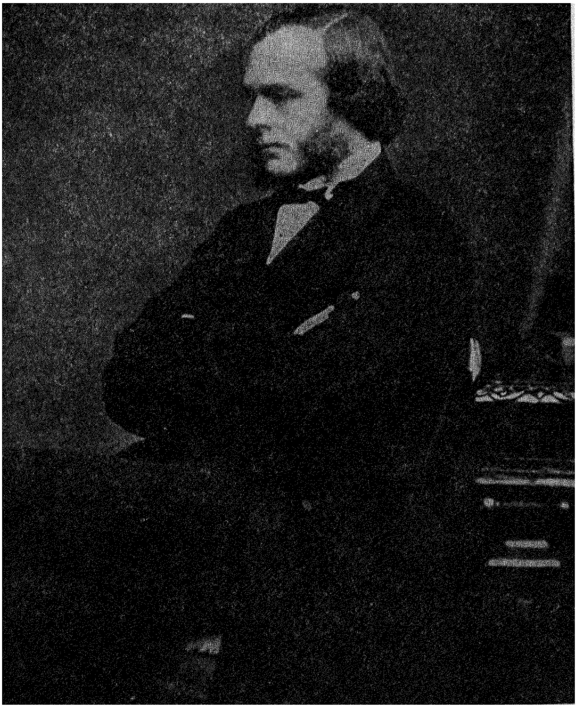

FIG. 4. Joseph Lister, 1827–1912.

"Lister's antiseptic system." Although today the details of the methods used are modified, the principle that Lister established is still adhered to and it forms the very cornerstone of surgical and obstetrical practice. The principle is that *micro-organisms must be kept out of wounds.* Lister suffered the criticism which seems to be the lot of all pioneers, but by 1875 he was internationally famous and honored everywhere. He died in 1912.

The result of the introduction of antiseptic methods into surgery and obstetrics was the seemingly miraculous disappearance of those serious complications which had previously followed any type of wound. Thus, countless lives were saved and untold suffering prevented. Antiseptic methods combined with ether anesthesia, introduced by Morton in the United States in 1846, have made possible the wonderful benefits of modern surgery. The work of Lister also powerfully stimulated the further study of germ diseases.

Louis Pasteur (1822–1895). Louis Pasteur (Fig. 5) was born in the village of Dole, France, in 1822, the son of humble parents, his father being a tanner. He began life with no special advantages, but became the most prominent man of his day, and is universally recognized as a genius whose brilliant achievements place him in the front rank of great men, and whose influence is still felt in many fields of science. He was originally trained as a chemist and at the age of 25 had already distinguished himself in this work. He never studied medicine, though his discoveries revolutionized medical practice. He was graduated from the Normal School in Paris in 1847, and taught at Strassburg and Lille, before coming again to Paris where he became professor at the Ecole des Beaux Arts and later (1867) at the Sorbonne. In 1888, in recognition of his incomparable achievements, a special laboratory, called the Pasteur Institute, was built for him in Paris by popular subscription. Acclaimed the world over for his epoch-making discoveries, Pasteur died in Paris, September 28, 1895.

Principal contributions of Pasteur. We have already described how Pasteur disproved the theory of spontaneous generation and demonstrated the true nature of fermentation. His studies in fermentation led him to the study of disease, first in animals, then in human beings.

In 1865, he was asked to attempt to find the cause of a disease of silkworms called pébrine, which was threatening to ruin the business of raising silkworms, an important industry in southern France. After several years of effort, Pasteur succeeded in demonstrating that pébrine is caused by a microscopic germ—a protozoan—and showed that the disease could be eliminated by choosing for breeding only those worms which were free of the parasite. This discovery was one more step toward the establishment of the truth of the germ theory of disease.

But the conclusive proof was furnished by the work of Pasteur and Koch with anthrax—a common disease of cattle and sheep which occasionally occurs in human beings. Rayer and Davaine as early as 1850, and Pollender in 1855, had seen little rods in the blood of animals dead of anthrax, and Davaine in 1863 reproduced the disease in healthy animals by inoculating them with blood containing these rods. It was left to Koch and Pasteur to demonstrate fully that these rods were bacteria and the cause of anthrax (1877). Pasteur grew the anthrax organisms by themselves in sterilized yeast water and kept them in the laboratory for several months, transferring them frequently to new culture fluid in which they multiplied readily, and showed that these cultures would always cause anthrax when inoculated into healthy animals. There could be nothing in these cultures but the bacteria themselves which could possibly be responsible for the production of the disease.

Koch, working independently in Germany about the same time, also cultivated the anthrax germs in pure culture, and showed that they produced disease in laboratory animals. He studied the growth and development of the organisms under the microscope, and for the first time demonstrated the real nature of the resistant bodies called spores, which the bacilli form. This careful work, taken with that of Pasteur, completed the proof that anthrax is caused by this organism and this organism alone. Anthrax was, therefore, the first disease of animals and man which was proven to be caused by a particular bacterium.

A contribution of still greater importance was made by Pasteur when he discovered and applied the principle of *protective inoculation or vaccination against disease.* In 1880 he was working on a disease of fowls called chicken cholera. He found that pure cultures of the germ of this disease, which had been kept in the laboratory for some time, would not kill his animals as fresh cultures did, but would merely cause a passing illness from which they recovered. Then he discovered that *the animals that had recovered from a previous inoculation of a weakened germ were*

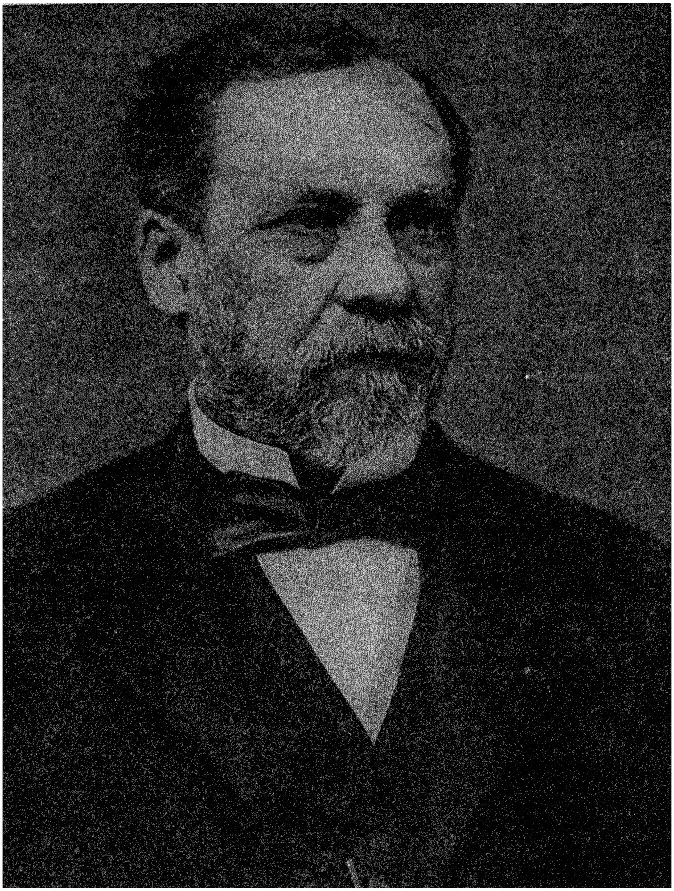

Fig 5. Louis Pasteur, 1822–1895.

immune and did not succumb to the disease when inoculated with a fresh culture, although other fowls were as susceptible as ever. Pasteur immediately perceived that there might be here a practical method of preventing disease. He saw that it might be possible to make individuals resistant to a disease by inoculating them deliberately with the weakened (and therefore harmless) germs of that disease.

The idea of protective inoculation was not entirely new, but the principle was not fully understood before Pasteur. One very important application of the idea was already well known. This was the method of vaccination against smallpox with the virus of cowpox. This great discovery had been made by the English physician Edward Jenner in 1798.

Pasteur first applied the principle of protective inoculation to the prevention of anthrax. He succeeded in making animals immune by a series of inoculations with anthrax germs whose disease-producing power had been so reduced that the injections caused no harm to the animals. The preparation of anthrax organisms used for these inoculations was called *anthrax vaccine.** In 1881, before a large audience of friends and critics, Pasteur demonstrated the value of his method. He had 50 sheep, 25 of which he had previously inoculated with his vaccine containing weakened anthrax germs; the other 25 were untreated. In the presence of his visitors he inoculated all 50 animals with a fresh culture of anthrax germs and invited everyone to return in two days to observe the result. When the company assembled again at the appointed time, everyone was moved to the most profound admiration, for all of the vaccinated animals were perfectly well, while of the unvaccinated animals 22 were already dead and the other three were dying. This method of protecting sheep and cattle from anthrax by use of a vaccine was soon tried out on a large scale and it is still used all over the world with great success.

The crowning achievement of Pasteur was the successful application of this principle of vaccination to the prevention of rabies, or hydrophobia, in human beings (1885). He was not able to find the germ of this disease under his microscope—we now know that the causative agent is an invisible virus—but he developed a system of inoculation with the weakened virus which prevents the development of this fatal disease if the inoculations are given soon after the bite of the rabid animal. Now this life-saving "Pasteur treatment," or a modification of it, is used everywhere for the prevention of hydrophobia.

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* The term "vaccine" had previously been applied only to the cowpox virus used by Jenner for immunization against smallpox. *Vacca* in Latin means *cow,* hence cowpox virus was called "vaccine virus," and Jenner's method of protective inoculation against smallpox was properly called *vaccination.* Pasteur, wishing to honor Jenner, deliberately extended the use of the term "vaccine" to indicate any preparation used like "vaccine virus" for immunization, and the word *vaccination* thus came to have the general meaning it has today.

Perfection of the compound microscope. The first microscopes really worthy of the name were those made by Leeuwenhoek about 1675. These were *simple microscopes,* that is, they contained only *one lens* or magnifying glass. They permitted Leeuwenhoek to see some of the larger micro-organisms. But bacteriology and other modern microscopical sciences depended for their development upon the perfection of *compound microscopes,* having *several lenses arranged in combination* and capable of very high magnification.

Early compound microscopes. The earliest compound microscopes were manufactured many years before the time of Leeuwenhoek. As early as 1600 a compound microscope was invented by a spectacle maker named Zacharias Jansen in Holland. The Italian scientist, Galileo, famous also for his improvement of the telescope, made a compound microscope about 1610, and it was to one of Galileo's instruments that the name microscope was first applied (1625). The early instruments were exceedingly crude, judged by the standards of today. They were really small inverted telescopes intended for observation of minute objects by reflected light. It was not until the end of the seventeenth century that microscopes were made to observe *transparent* objects by transmitted light as in the modern instrument.

During the eighteenth century the microscope was gradually improved by various workers in England, France, Italy, Germany and America, but for most of this time it was scarcely more than a kind of scientific toy, distorting more than it magnified. About 1830 important advances were made in the construction of the optical parts of the instrument, largely as the result of the principles worked out by Joseph Jackson Lister, father of the surgeon, Joseph Lister. But it was not until about 1880 that the microscope was converted into the beautiful instrument of precision and power we have today.

The modern microscope. We owe the excellence of the modern microscope almost entirely to the work of one man, Ernest Abbe (1840–1905). Abbe was a German physicist who became connected in 1866 with the firm of Zeiss, manufacturer of microscopes in Germany. He made fundamental improvements in the entire optical system of the microscope, including the introduction of superior oil-immersion objectives, and the Abbe substage condenser. The perfection of the compound microscope, with its powerful lenses magnifying a thousand times, was the one thing necessary to assure the rapid development of the new science of bacteriology.

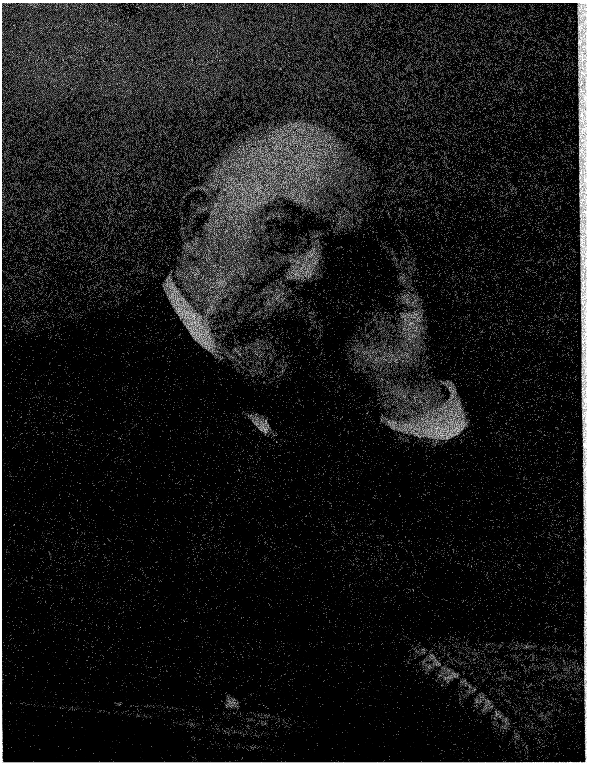


Fig. 6. Robert Koch, 1843–1910.

Robert Koch (1843–1910). Robert Koch was born December 11, 1843, in Germany (Fig. 6). He took his degree in medicine in 1866 and began a general practice in a small country town. He became much interested in microscopical studies and soon these absorbed most of his time. On April 22, 1876, Koch addressed a letter to Ferdinand Cohn, professor of Botany at Breslau, the nearest university town, in which he reported that he had made a study of the anthrax germ. Cohn invited the unknown country physician, Koch, to Breslau, and gave him the opportunity of demonstrating his specimens and methods. His studies were so complete and well ordered that they made a profound impression, at once marking him as a new leader in bacteriology. The work on anthrax was published the same year and soon Koch's name became well known throughout the medical world. In 1880 Koch was called to the Imperial Health Department at Berlin and later became Professor of Hygiene, and Director of the Institute for Infective Diseases. Many years of his later life he spent abroad in the study of cholera, malaria, and other diseases. He died May 27, 1910.

Principal contributions of Koch. Aside from his work with the anthrax germ already mentioned, Koch is especially noted as the first to master the technical difficulties in the study of microorganisms. It was Koch who improved and simplified methods of studying bacteria and thus made it possible for persons of ordinary skill to obtain entirely satisfactory results. This improvement of methods is the greatest single factor which accounts for the phenomenally rapid development of bacteriology during and since Koch's time. Koch introduced the method of making smears of bacteria on glass slides, and of staining them with the anilin dyes, first used a few years earlier by Ehrlich and Weigert. He also was the first to employ in bacteriological work the improved compound microscope of Abbe.

In 1881 Koch devised a method of growing bacteria in a solid culture medium made with gelatin, and introduced his plate method of securing pure cultures. The use of a liquefiable solid medium was a new idea. Vegetable infusions and other liquids had been used almost exclusively up to this time. It was naturally very difficult to separate out one kind of bacteria from a mixture of organisms in a liquid medium and to get it growing by itself in a pure culture. Koch's solid culture medium plate method made the task of securing pure cultures comparatively simple and certain, and immensely facilitated the study of individual kinds of bacteria.

He inoculated the gelatin with a mixture of bacteria after the medium had been melted by warming, then poured the inoculated medium upon a sterilized glass plate to harden. Wherever the bacteria were caught in the solidified medium they multiplied and soon formed a mass, called a *colony*, visible to the naked eye. If the organisms originally inoculated were well separated from each other in the medium, the colonies they formed would be pure, that is, the colonies would contain only one kind of bacterium. To secure a pure culture it was only necessary, then, to pick out such a colony with a sterile wire and transfer it to a tube of fresh sterile medium. This method of obtaining pure cultures is essentially that used today, except that *agar* is used instead of gelatin for this purpose, and an especially devised glass dish is employed for our "plate" cultures, called the *Petri dish*, introduced by Petri in 1887.

These new and better methods permitted Koch and his followers to discover the germs of several important diseases almost at once. Koch himself described several of the organisms found in wound infections (1878), and the spirillum of cholera in 1883.

In 1882 Koch startled the world when he announced the discovery of the germ of tuberculosis. He was able to demonstrate in a most convincing manner that all forms of this dread disease are due to the same cause—the tubercle bacillus (*Mycobacterium tuberculosis*). He described a special staining method for detection of this germ and grew the organism in pure cultures in the laboratory. He showed that animals would develop tuberculosis when inoculated with pure cultures of this organism and he recovered the identical germs from the diseased tissues of the animals. In his full report on these studies (1884) Koch expounded his postulates or laws by which an organism may be proven to be the cause of a particular disease (see p. 16), and he showed how he had fulfilled these laws in his own work on the causation of tuberculosis. This brilliant work of Koch gave mankind for the first time a true understanding of one of the most common and deadly of human ills, and furnished the basis for practical preventive measures. It is a shining landmark in the history of medicine.

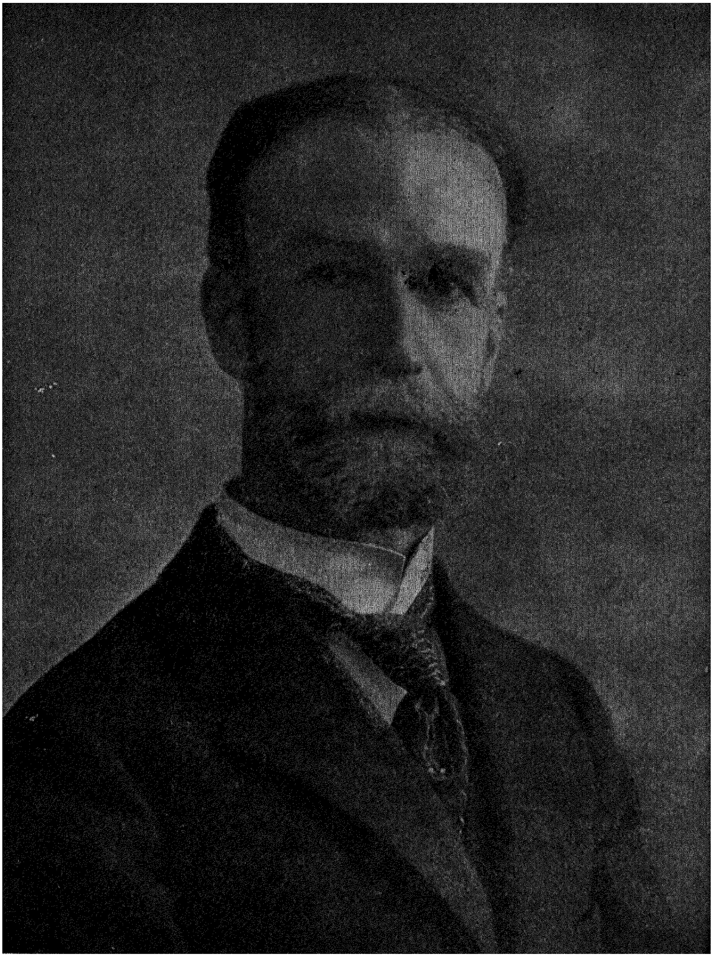
Koch made one other important contribution when he discovered *tuberculin*, a substance in cultures of tubercle bacilli that causes a specific reaction when injected into a tuberculous individual.

THE MODERN PERIOD, 1882 TO THE PRESENT DAY

The great contribution of Pasteur and Koch to the development of bacteriology cannot be measured merely by the discoveries they themselves were able to make. Their influence in stimulating and directing the work of others must be counted as of nearly equal importance. About each of these leaders there developed a group of younger workers, and in America as well as in Europe many able investigators began to study bacteria intensively. One of the pioneer American bacteriologists was George M. Sternberg (1838–1915), an Army physician who became Surgeon General. Other early American bacteriologists who have attained worldwide fame are William H. Welch (1850–1934) of Johns Hopkins University, and Theobald Smith (1859–1934) of the Rockefeller Institute (Fig. 7).

Within twenty years following 1882, all but a very few of the disease-producing bacteria, protozoa, and fungi we now know were identified. Many of the most important discoveries were made by associates or pupils of Pasteur or Koch. By 1910 other types of infectious agents—spirochetes, rickettsiae, and filtrable viruses—had been recognized. It was also very early in this period that the fundamental observations and studies were made which founded the now very complex subject of *immunology*. This rapidly increasing store of knowledge was soon applied to the solution of many of the practical problems of the farmer and dairyman, and with particularly marked success to the diagnosis, prevention, and treatment of germ diseases.

Discovery of important disease germs. It was natural that the larger organisms should be discovered first. It is not surprising to learn, then, that some of the important fungi and protozoa were recognized years before the modern period of microbiology began. For example, several of the types of fungi responsible for ringworm of the skin were described before 1850; the protozoan (ameba) causing amebic dysentery was discovered in 1875, and the germ of malaria (another kind of



Theobald Smith III/27/21

1859 - 1934

Fig 7. Theobald Smith, 1859–1934. This splendid portrait, with autograph, of the great American bacteriologist is reproduced through the courtesy of Professor Simon Henry Gage, Cornell University.

protozoan) was first seen by Laveran in 1880. With the development of better technical methods under the influence of Koch there ensued a "golden age of discovery"—more disease-producing protozoa and fungi were soon found, and there was an especially rapid advance in the recognition of the bacteria that cause common diseases.

The following list shows when, and by whom, some of the more important disease-producing bacteria were discovered.

Actinomyces bovis, mold-like "higher" bacterium causing *actinomycosis,* Langenbeck, 1845, Hartz and Bollinger, 1877.

Borrelia recurrentis, spirochete of *relapsing fever.* Obermeier, 1873.

Bacillus anthracis, germ of *anthrax.* Rayer and Davaine, 1850, Pollender, 1855. Proved to be cause of anthrax independently by Koch and Pasteur, 1877.

Mycobacterium leprae, germ of *leprosy.* Hansen, 1874.

Gonococcus (Neisseria gonorrheae), germ of *gonorrhea.* Neisser, 1879, Bumm, 1885.

Pneumococcus (Diplococcus pneumoniae), germ of *lobar pneumonia.* Pasteur, Sternberg, 1880.

Eberthella typhi, germ of *typhoid fever.* Eberth, 1880, Gaffky, 1884.

Staphylococcus, germ causing *abscesses.* Pasteur, 1880, Ogston, 1881, Rosenbach, 1884.

Streptococcus, germ causing *acute infections of various tissues, including septicemia and erysipelas.* Koch, Ogston, 1882, Fehleisen, 1883, Rosenbach, 1884.

Mycobacterium tuberculosis, germ of *tuberculosis.* Koch, 1882.

Loefflerella mallei, germ of *glanders.* Loeffler and Schütz, 1882.

Vibrio cholerae, germ of *Asiatic cholera.* Koch, 1883.

Corynebacterium diptheriae, germ of *diphtheria.* Klebs, 1883, Loeffler, 1884.

Clostridium tetani, germ of *tetanus.* Nicolaier, 1884, Kitasato, 1889.

Meningococcus (Neisseria meningitidis), germ of *epidemic meningitis.* Weichselbaum, 1887.

Clostridium welchii, principal germ in cases of *gas gangrene* Welch and Nuttall, 1892.

Pasteurella pestis, germ of *plague.* Kitasato, Yersin, 1894.

Brucella abortus, one of the germs causing *undulant fever (brucellosis)*. Bang, 1895.

Shigella dysenteriae (Shiga), a germ causing *bacillary dysentery*, Shiga, 1897.

Shigella paradysenteriae (Flexner), another variety of *dysentery* germ, Flexner, 1900.

Treponema pallidum, the spirochete causing *syphilis.* Schaudinn and Hoffman, 1905.

Hemophilus pertussis, germ of *whooping-cough.* Bordet and Gengou, 1906.

Bacterium tularense, germ of *tularemia.* McCoy and Chapin, 1910.

Streptococcus scarlatinae, germ of *scarlet fever.* Savchenko, 1905, Gabritschewsky, 1907, Dick and Dick, 1923.

Discovery of filtrable viruses. Early in the period of most active discovery, investigators attempted to separate bacteria from the culture fluids in which they were growing by filtration, in order to study the properties of the filtrates. Chamberlain, in 1884, devised a cup of unglazed porcelain which proved to be the first successful bacterial filter. When a broth culture in which bacteria were suspended was passed through this filter all of the organisms were held back, and the filtrate was clear and sterile. Study of the filtrates obtained from cultures of various bacteria soon led to a most important finding—some of these organisms, notably the germs of tetanus and of diphtheria, were shown to produce powerful poisons (*toxins*) which appeared in the sterile filtrate.

Then came the unexpected discovery that the actual causative agent of some of the communicable diseases will pass through a bacterial filter in invisible form. In 1892 Iwanowski proved that the mosaic disease of tobacco leaves could be transferred to healthy young plants by the *filtered* juice from the diseased leaves, though this filtrate contained no microbes recognizable under the microscope. In 1898 Loeffler and Frosch discovered that foot and mouth disease is also caused by a filtrable, invisible "virus." Soon, many other diseases of this kind were recognized in both animals and man.

Among important human diseases found to be caused by viruses are measles, mumps, influenza, smallpox, rabies, poliomyelitis, and encephalitis.

Discovery of rickettsiae. Still another class of infectious agents was discovered by Ricketts in 1909–10, when he discerned extremely minute bacteria-like bodies in the blood of patients ill with Rocky Mountain spotted fever and typhus fever, and in the lice that had fed on typhus patients. These bodies, now called *rickettsiae*, have since been proved to be living organisms. They are set apart from other organisms in a class by themselves because, although they are visible under the microscope, they will not grow in artificial media like the ordinary bacteria.

The two diseases mentioned above are the principal ones caused by the rickettsiae and the only rickettsia infections ordinarily occurring in the United States.

Modification of Koch's postulates. The investigators who made these discoveries were not always able to carry out completely Koch's rules for proving the relation of the organisms they studied to the causation of the disease in which they found them, largely for the reason that it was often difficult, and in some cases impossible, to reproduce the typical diseases in laboratory animals. However, evidence of a new kind was used. It was found that the blood of a diseased individual will act in a definite way upon the germ causing the disease, and when properly tested will act upon this germ alone. Thus the guilty germ could be identified by tests of the patient's blood.

On one other point the original postulates of Koch have been modified. These postulates state that a germ cannot be regarded as the cause of a disease if it is found in healthy persons. We now know that this is a mistaken idea. Diphtheria germs, for example, are often found in the throat of a healthy person, yet these same organisms may cause severe diphtheria if they lodge in the throat of another, more susceptible individual.

Development of immunology. A most important step toward the understanding of resistance (immunity) to disease was made by Elie Metchnikoff (1845–1916), a Russian scientist, who worked in Pasteur's laboratory (Fig. 8). In 1882–1884 he described his studies on the rôle of the white cells (leukocytes) of the blood in defending the body against disease germs. He showed that these cells, which he called *phagocytes*—or "devouring cells"—were able to take into their own substance foreign bodies of various kinds, *including disease germs.* In some cases the germs were actually destroyed—dissolved—within the leukocytes, or were carried away from the inflamed tissues by them. Metchnikoff believed that the engulfing of bacteria by the white blood cells (the process called *phagocytosis*) was the principal defense the living body possesses against the invasion of disease-producing germs.

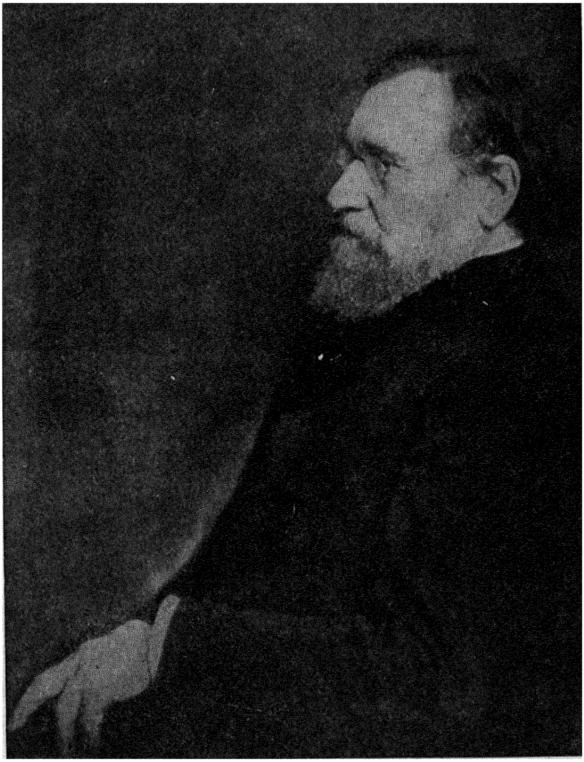


Fig. 8. Elie Metchnikoff, 1845–1916.

(1854–1915), a German scientist and associate of Koch, who explained immunity on the basis of chemical properties of the blood (Fig. 9). These conflicting views of immunity stimulated a great deal of experimental work which still continues. As a result of these investigations, we now have a vast amount of detailed information about the reaction of the body to disease germs, though as yet by no means a complete understanding of all the complex phenomena associated with immunity. One practical result of immunological studies was the development of valuable *blood tests for the diagnosis of germ diseases,* as, for example, the *Wassermann test* for syphilis, and the *Widal test* for typhoid fever.

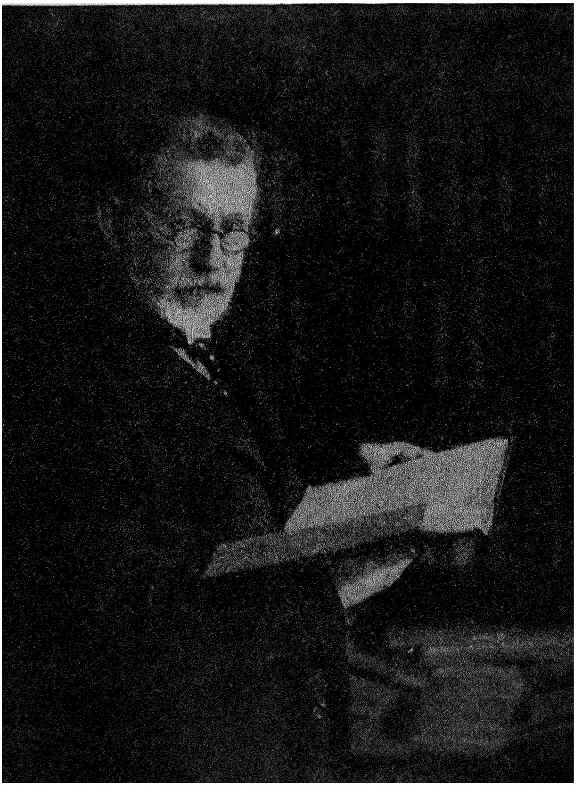


FIG. 9. Paul Ehrlich, 1854–1915.

Another application of immunology was the development of *successful methods of protective inoculation or vaccination* against a number of important germ diseases. Vaccination against *typhoid fever,* introduced into the English army by Wright in 1896 and made compulsory in the American Army in 1912, has apparently been most successful in preventing this disease, as shown by the very few cases of typhoid among vaccinated troops in the World War. Vaccination against *diphtheria* by use of toxin-antitoxin mixtures, first put into practice by Behring in 1912, and more recently the use of *toxoid*, introduced by Ramon in 1925, has proven to be a measure of immense importance in the control of diphtheria.

The greatest practical advance was the *discovery of antitoxin.* Kitasato and Behring, in connection with their studies of the tetanus germ (1890), found that the blood of animals which had been made immune to the poison (toxin) of that germ contained something which neutralized the poisonous action of the toxin. This substance in the blood of immune animals which neutralized the tetanus toxin, they called *tetanus antitoxin.* If blood containing this antitoxin from an immune animal was inoculated into a normal animal, this second animal was protected against tetanus. A short time later Behring showed that it is possible to prepare an antitoxin which will cure diphtheria, and soon these antitoxins were being prepared on a large scale. The world wide use of diphtheria antitoxin, which has saved countless lives, dates from 1894.

Other important advances in immunology included the discovery of the human *blood groups* by Landsteiner (1901) which led to the universal practice of "matching" the blood of patient and donor before transfusions, and of the phenomenon of *hypersensitivity* toward foreign substances. Studies of the latter have given us an insight into the nature of a whole group of common ills, examples of which are hay fever and asthma, that are caused not by microbes, but by sensitiveness toward plant pollens or other foreign matter.

Applications of microbiology and immunology in public health work. The influence of these many discoveries upon public health work has been truly revolutionary. Before the rise of microbiology, diseases were thought to be carried by some mysterious effluvium which polluted the atmosphere and against which we could have no protection. Epidemics of contagious diseases were thought to arise under the influence of the stars, or were attributed to storm or earthquake. The source of disease was always thought of as existing *outside* of the bodies of men and animals, and filth of any kind was especially feared.

But microbiology has taught that the diseases which pass from man to man or from animals to man are caused by certain particular micro-organisms that live and grow in the bodies of men and animals and, with rare exceptions, do not have any natural life anywhere else. The secretions and excretions of individuals who are sick, and sometimes of individuals who are well, contain these organisms, and it is when these germ-laden materials are transferred more or less directly from one individual to another, that disease spreads. This knowledge has made clear the value of the isolation of the patient (or carrier of the organism) and prompt destruction of the germs in the discharges from the bodies of these persons.

Exact knowledge of the germs concerned has made possible the development of accurate laboratory tests for the diagnosis of disease and led to the establishment of diagnostic laboratories everywhere.

Microbiology has taught the parts played by the soil, water, sewage, milk, and insects in the transfer of disease germs, and special methods of protection have been developed.

Microbiology has taught not only how to grow germs, but how to exclude and avoid them, and so has furnished a rational basis for personal hygiene.

Immunologists have developed highly successful methods for increasing the resistance of individuals to certain diseases by inoculation of vaccines or the blood serum of immunized animals, and by these means the number of cases and deaths from typhoid fever, diphtheria, and other diseases have been greatly reduced.

REVIEW QUESTIONS—CHAPTER III

1. Outline briefly the life of Joseph Lister.
2. Describe what Lister did to prevent the inflammation of wounds. What is the principle behind his antiseptic methods? What is the importance of Lister's work in the history of medicine?
3. Outline briefly the life of Louis Pasteur.
4. Describe Pasteur's work on silkworm disease.
5. Describe Pasteur's studies of the cause of anthrax. What importance has this work?
6. How did Pasteur discover the principle of protective inoculation (or vaccination) against disease?
7. Who had already applied this principle for protection against smallpox, and when? Explain the origin and present meaning of the terms vaccine, vaccination.

8. What did Pasteur's "anthrax vaccine" contain? Explain why the germs in the vaccine were weakened. How did Pasteur demonstrate the value of the vaccine for the prevention of anthrax?
9. For what other disease did Pasteur successfully develop a system of protective inoculations?
10. Define (*a*) simple microscope, (*b*) compound microscope.
11. Who made the first good simple microscopes?
12. Outline the story of the invention and improvement of the compound microscope to 1880.
13. What did Ernest Abbe contribute to the perfection of the modern microscope?
14. Outline briefly the life of Robert Koch.
15. What was Koch's contribution to the study of anthrax?
16. How did Koch improve microscopical methods?
17. Describe Koch's plate method of isolating pure cultures. Explain why the introduction of liquefiable solid culture media and the plate method was an important advance in bacteriology.
18. Name important disease germs which Koch discovered.
19. Describe Koch's work on tuberculosis and discuss its importance.
20. In addition to their own discoveries, what contribution did Pasteur and Koch make to the advancement of bacteriology?
21. Name three early American bacteriologists.
22. Name the bacteria causing some important diseases and tell when and by whom the germs were discovered.
23. Describe the discovery of filtrable viruses. Name several virus diseases of human beings.
24. Who discovered the rickettsiae, and when? What two human diseases are caused by these organisms in the United States? What distinguishes the rickettsiae from ordinary bacteria?
25. How and why have Koch's laws been modified?
26. Who was Elie Metchnikoff? Outline briefly his theory of immunity. Who opposed his view and what was the effect of the conflicting ideas on the development of immunology?
27. Mention five important practical advances in immunological studies.
28. Mention five ways in which microbiology has been of help in public health work.

CHAPTER IV

GENERAL PROPERTIES AND CLASSIFICATION OF MICRO-ORGANISMS

IT IS SOMEWHAT difficult to realize, until one gets used to the idea, that the extremely tiny, invisible microscopic organisms to be studied in microbiology are living, independent organisms, —just as much alive as a tree, or a bear, or a man. They possess all the essential properties of life, though in elemental form, and all of their activities and characteristics result from the fact that they are alive.

In order to understand micro-organisms, therefore, it is necessary to know some of the general properties of living things.

SOME GENERAL CHARACTERISTICS OF LIVING THINGS

Living matter is made up of microscopic units called cells. One of the most outstanding peculiarities of living substance is revealed when we examine its intimate structure under a microscope. No matter how diverse the outward form may be, we find that the bodies of all plants and animals are made up of well defined microscopic units called *cells.*

These tiny cells were first named by Robert Hooke (1665). He examined a section of cork with a microscope and saw that it was made up of "little boxes or cells distinct from one another." As Hooke saw them, each cell was an empty chamber surrounded by a solid wall. But the cork which Hooke described was really only the lifeless remains of the living tree bark from which it came and all the living substance in it had dried up and disappeared. The true living matter of the cork had filled these cork cells during the life of the bark. The term *cell* is, therefore, not really appropriate, though we still use it, because *the essential part of a living cell is not the wall,* which may be very thin indeed, or made up of lifeless matter as in cork, *but the contents within the wall.*

The fact that all living matter is composed of microscopic cells was first demonstrated convincingly by Schleiden and Schwann in 1838 and the idea that cells are the units of structure and also of function in living bodies has been an accepted tenet in biology since the work of the great German pathologist Rudolph Virchow (1860).

Larger plants and animals are multicellular (many-celled). The larger, more complex living things, plants or animals, are many-celled or *multicellular organisms.* They are composed of great numbers of cells, which are combined into various *tissues,* each made up of cells of a different type, such as muscle cell, nerve cell, bone cell, etc. These tissues are combined to form the various *organs* of the body, as the muscles, brain, lungs, liver, heart, and the rest.

Most microbes are unicellular (one-celled). But the bacteria and most of the other micro-organisms are one-celled or *unicellular organisms.* They occur as single cells, each cell being an independent individual, having within itself all the properties of life (Fig. 27). The entire body of a bacterium is, then, a single living cell.

Protoplasm. All living cells (that is to say, all the living parts of the bodies of microbes or men, plants or animals) are composed of a substance called *protoplasm* (from the Greek words, meaning *first-formed substance*). By protoplasm is meant the elemental living matter composing the bodies of living things. So far as the essential characteristics which make it alive are concerned, protoplasm is the same in every form of life. The properties of protoplasm are the fundamental properties of life, for protoplasm is the physical basis of life.

When protoplasm is observed in its most nearly pure state, that is, unmixed with nonliving matter, it appears as a clear, somewhat sticky, semiliquid substance. It is always liquid, though sometimes quite jelly-like, and is, in fact, composed very largely of water. But, in addition, a very great many different substances of very complex chemical makeup enter into the composition of protoplasm. Even in so simple an organism as a bacterium, the protoplasm is a very complex mixture, made up of many substances suspended in the semiliquid in a very finely divided state and emulsified one within the other. Living matter differs *chemically* from nonliving matter chiefly in the extraordinary complexity of its internal composition and the variety and complicated nature of its reactions.

Structure of cells. There is a great deal of difference in the size, shape, and structure of the various types of cells which make up the bodies of multicellular plants and animals, and also there are differences among single-celled micro-organisms. Certain structures are found, however, in nearly all kinds of cells (Fig. 10).

Every cell, of whatever kind, is a microscopic protoplasmic mass with a well defined outline and a more or less rigid shape. Some cells, as those making up most plants, have a relatively thick *cell wall*, but most cells in animal bodies do not show a definite surrounding wall. *The bacteria and most other unicellular organisms have only very thin cell walls and are really little more than naked masses of protoplasm.*

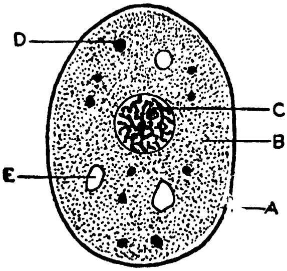


FIG. 10. Diagram of a cell. A, cell wall or membrane; B, cytoplasm; C, nucleus; D, particle of food or other nonliving material suspended in the cytoplasm; E, a vacuole.

In every cell, part of the protoplasm is differentiated into what is called the nuculear material, and this material seems to control the activities of the entire cell. In most plant and animal cells, the nuclear material is collected into a central body called the *nucleus.* This nucleus will take a deeper color, when stained with certain dyes for examination under the microscope, so that it stands out clearly from the rest of the cell substance, which is called the *cytoplasm.*

Bacterial cells differ from almost all other living cells in that they do not possess definite nuclei, except in the case of a very few kinds. In most bacteria, the nuclear material is intimately mingled with all of the protoplasm, so that the entire bacterial cell usually takes a stain like the nucleus of a plant or animal cell.

Vital functions of living cells. Every living organism, including *each individual bacterium or other micro-organism,* exhibits certain distinctive properties which are not possessed, or at least are not all possessed, by any lifeless thing. Physiologists usually mention five properties which are characteristic of living organisms, namely: (1) movement, (2) growth, (3) reproduction, (4) metabolism, and (5) irritability.

We instinctively recognize *the power to make movements* as a characteristic property of a living thing. Among micro-organisms, there are many kinds which are able to travel from place to place, as animals do; others cannot do this, but they show limited movements during the processes of growth and reproduction.

A second property is *the capacity to grow,* to increase in body substance by growth from within.

A third vital function of living organisms is *the capacity to reproduce* their own kind and so to multiply in numbers. The possession of this property of reproduction is regarded as proof that a thing is alive, even though we know nothing more about it.

A fourth property of living things is *the capacity to carry on a continuous interchange of materials with the air and with food substances*—a process called *metabolism.* Every living thing takes in food, liberates energy, and excretes waste products so long as it remains alive. Every living organism *respires* continuously, that is, it takes in oxygen, consumes it, and gives off carbon dioxide.

Finally, all organisms show *irritability,* that is, they respond to stimuli, and react in some way to changes in their environment.

Classification and naming of living organisms. Different kinds of living things show a different character or degree of movement, grow at different rates and under different conditions, reproduce by different methods, carry on a different set of complicated chemical interreactions with their surroundings, producing distinctive metabolic products, and react in different ways to various external influences. On the basis of these differences, as well as the form and size of the organism, living things are placed in either the *plant kingdom* or the *animal kingdom.* They are then further classified into groups and subgroups in the following order:

(1) *Phylum*
(2) *Class*
(3) *Order*
(4) *Family*
(5) *Tribe*

(6) *Genus*
(7) *Species*

The scientific name of any kind of organism is written in Latin and italicized, and is made up of two elements: (1) the name of the *genus* spelled with a *capital* letter, followed by (2) the name of the *species* spelled with a *small* letter. Thus, among bacteria, for example, we have the names *Bacillus subtilis*, *Staphylococcus albus*, *Staphylococcus aureus*. In each case the first word gives the genus to which the organism belongs, the second gives the species name.

TYPES OF MICRO-ORGANISMS AND THEIR CLASSIFICATION

The seven principal types of microbes. As we have already learned, the seven principal groups of micro-organisms are called (1) *protozoa*, (2) *true fungi*, (3) *mold-like higher bacteria*, (4) *spirochetes*, (5) *true bacteria*, (6) *rickettsiae*, and (7) *filtrable viruses*. In the following paragraphs we shall try to give a general notion of what these microbes are like, and how they are related to one another.

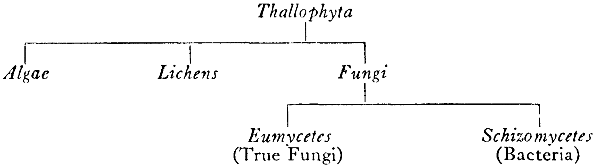
Size and form. An idea of the size and form of different kinds of microbes (except the viruses, which we do not attempt to picture) may be gained from an inspection of Fig. 11. Here are illustrated some typical members of each of the classes and sub-groups mentioned below.. The drawings are made to scale, and a scale is given in *micra*. The unit of measurement used to express the size of micro-organisms is the *micron* (μ), plural *micra*. One micron is equivalent to 1/1000 of a millimeter, or about 1/25,000 of an inch.

Protozoa. Largest and most complicated in structure and mode of life are the microbes comprising the phylum *Protozoa* (first or simplest animals). This is the only group of micro-organisms which clearly belongs in the *animal kingdom*. The intimate study of these microbes is the separate science of *Protozoology*. As indicated, there are four classes of protozoa: (1) the *Sarcodina* (of which the principal subgroup is the *Amebae*), (2) the *Mastigophora* (or *Flagellates*), (3) the *Infusoria* (or *Ciliates*), and (4) the *Sporozoa*.

The examples pictured (Fig. 11: 1, 2, 3, 4) show pathogenic organisms of each class, namely: (1) the ameba responsible for amebic dysentery, (2) a flagellate (trypanosome) that causes African sleeping sickness, (3) a ciliate that sometimes produces a mild intestinal disease, and (4) some forms of the parasites of human malaria, the most important of the pathogenic sporozoa. (Chapter V).

The plant-like microbes; bacteria and true fungi. Scientists have decided that all the other micro-organisms are most appropriately classed in the *plant kingdom.* This decision is based principally upon the fact that these microbes, in contrast to the typical protozoa, do not eat food particles and then digest them within the body as animals do, but instead, absorb food substances in solution through their cell walls, like the familiar, larger plants.

The two great classes among these plant-like microbes are the *Bacteria* (*Schizomycetes*), and the *True Fungi* (*Eumycetes*). The following diagram shows how these main groups fit into the plant kingdom:



The phylum *Thallophyta* contains those primitive plants that consist merely of a plant body (called a *thallus*), without differentiation into leaves, stems, or roots. The *Thallophyta* are divided into two main groups: (1) the *Algae,* which are provided with chlorophyll, like the familiar, higher green plants, and (2) the *Fungi,* which are colorless and devoid of chlorophyll.

Lichens are peculiar vegetations consisting of algae and fungi living together. They are commonly seen as scale-like growths covering tree trunks or rocks.

The most familiar *Algae* are those tiny, green or brown plants which are often seen forming a scum over the surface of a quiet pond. Many of the algae are microscopic and are shaped very much like some of the bacteria. They are considerably larger, however, and are colored green, like the familiar plants, whereas bacteria are colorless. The seaweeds are a form of algae.

To the *Fungi* belong all of the many varieties of lowly vege-

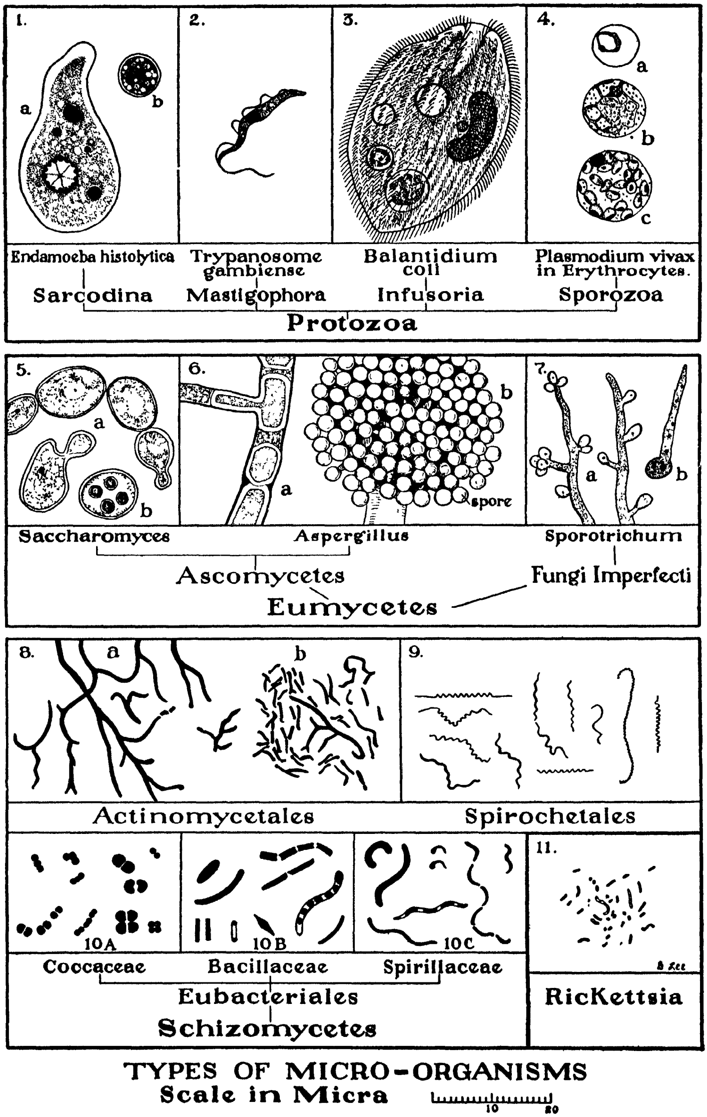
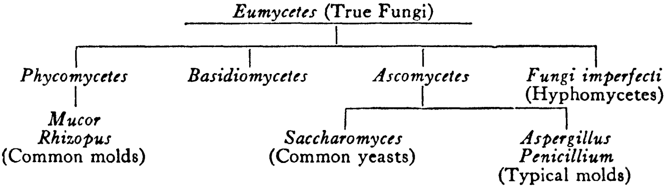


FIG. 11. Legend is on opposite page.

table organisms which lack chlorophyll. Some of these plants, such as the common mushroom, are large and visible to the naked eye. The majority are microscopic, however, and among them are the two groups of micro-organisms in which we are interested, namely, the so-called true fungi and the bacteria.

True Fungi (Eumycetes). These comprise the order of microbes ordinarily referred to when we use the term *fungi* without qualification. Their detailed study is the science of *Mycology*. A disease caused by a true fungus is called a *mycosis*, or a *mycotic disease* (Chapter VI).

The *Eumycetes* are subdivided as indicated in the following diagram.


Eumycetes (True Fungi)
Phycomycetes
Basidiomycetes
Ascomycetes
Fungi imperfecti (Hyphomycetes)
Mucor *Rhizopus* (Common molds)
Saccharomyces (Common yeasts)
Aspergillus *Penicillium* (Typical molds)

Included here are the common *yeasts* (*Saccharomyces*), such as those in a yeast cake. These organisms are single, oval, nucleated cells, varying greatly in size, but usually having a diameter of from 10 to 20 micra (Fig. 11:5). Also among the familiar true fungi are the common *molds*. Those of the genera *Aspergillus* and *Penicillium* grow as branched, intertwined filaments, made up of a succession of cylindrical, nucleated cells, strung end to end. These organisms, then, are really multicellular. They produce masses of reproductive spores (Fig. 11:6). The molds in the genera *Mucor* and *Rhizopus* grow similarly, but the threads are without cross walls. The network of branched filaments composing the body of molds, and the spore-masses,

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FIG. 11. Types of micro-organisms, drawn to scale. 1a, the trophozoite or growing form; 1b, the cyst; 4a, early signet-ring form of the protozoan within the red blood cell; 4b, later stage in the development of the parasite, accompanied by enlargement and stippling of the erythrocyte; 4c, final stage just before the cell bursts releasing the many small forms of the parasite (merozoites) which may then enter other red blood cells, thus continuing the asexual cycle in the human blood; 5a, budding of yeast cells; 5b, an ascus containing ascospores; 6a, portion of a branching filament and 6b, part of the "head," or fruiting body, of a common mold; 7a, the mature fungus with conidia (reproductive spores) developing from the filaments; 7b, a new filament growing out from a spore; 8a, filamentous growth of Actinomyces; 8b, fragmentation of the filaments into bacteria-like rods.

are visible to the naked eye, although the individual threads and spores are still of microscopic size, usually having a diameter of 2–5 micra.

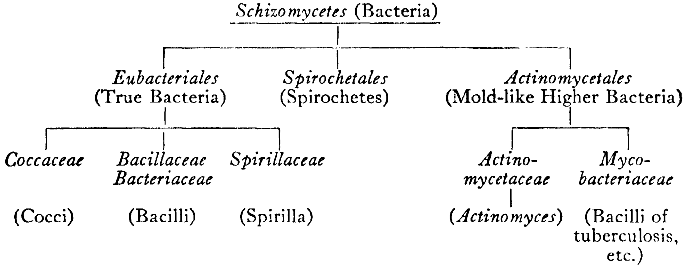
Among the *Basidiomycetes* there are some important plant parasites, but no species causing infection in human beings.

Most of the fungi pathogenic for man are found in the class called *Fungi imperfecti* (or *Hyphomycetes*). Some of these, such as *Sporotrichum*, the cause of sporotrichosis, have a filamentous structure similar to that of the ordinary molds (Fig. 11:7).

Bacteria (Schizomycetes). All the forms of bacteria, including the spirochetes and the mold-like higher bacteria, as well as the true bacteria, are included in the *Schizomycetes.* This word means, literally, "fission-fungi," and is appropriate because bacteria multiply by a process called simple fission—a splitting of an organism across its short axis to make two new organisms.

As shown in a diagram above, the *Schizomycetes* form a subdivision of the *Fungi.* In a strict botanical sense, then, bacteria might be called "fungi." But the majority of bacteria are so much smaller, and simpler in structure and mode of development, than typical molds and yeasts that they are generally thought of as an entirely separate group, and *the term fungi as commonly used does not include bacteria*, but refers only to the molds and other more complex micro-organisms belonging to the *Eumycetes.*

The principal orders and families of the *Schizomycetes* are shown in the following scheme:



Spirochetes (Spirochetales). Common species of spirochetes (Fig. 11:9) are delicate, flexible, coiled threads, suggesting microscopic snakes. They seem to be intermediate forms between the protozoa and the true bacteria. Syphilis is caused by a kind of spirochete (Chapter VII).

Mold-like Higher Bacteria (Actinomycetales). Typical organisms among the so-called *mold-like higher bacteria* (*Actinomycetales*) are those in the genus *Actinomyces*, which cause actinomycosis in cattle and in human beings. These grow as branched threads, like the true molds, but the individual filaments are much finer, and there is a marked tendency for the filaments to fragment into short, rod-like elements, resembling the ordinary bacteria (Fig. 11:8).

In the family *Mycobacteriaceae* are placed certain organisms which are much less mold-like than the *Actinomyces*, yet are somewhat more complicated in structure or mode of life than the ordinary bacteria. The limits of this special group cannot be sharply defined, and we mention it only because most authorities classify here several of the most common and most important disease-producing organisms, including the germ of tuberculosis and of diphtheria (Chapter VII).

True Bacteria (Eubacteriales). The *true bacteria* are the most numerous in kind, most conspicuous, and best known, of all the microbes. The majority of the harmless organisms that surround us everywhere, as well as the germs of important diseases, belong here.

The principal families are differentiated mainly on the basis of the characteristic shape of the bacterial cell. The *Coccaceae* are shaped like a ball or sphere, and are called **cocci** (singular *coccus*), the *Bacillaceae* and the *Bacteriaceae* are rod-shaped or cylindrical, and are called **bacilli** (singular *bacillus*), while the *Spirillaceae* are rigid spiral forms, called **spirilla** (singular *spirillum*) (Fig. 11:10,a,b,c.) (Chapters VIII, IX).

Rickettsiae and **filtrable viruses** cannot now be classified in any definite relationships to the other microbes, for we do not know just where they fit into the scheme of microbic life. The many-shaped *rickettsiae* (Fig. 11:11) are just within the limits of microscopic vision. They have a length or diameter of only about 0.2 or 0.3 μ (Chapter VII).

CLASSIFICATION OF MICRO-ORGANISMS ACCORDING TO THEIR DISTRIBUTION AND ACTIVITIES

Distribution of microbes: their place in nature. Microbes of some kind may be found in every spot where life is possible. They are abundant not only in the soil, in natural waters, and on vegetation everywhere in nature, but also in the dust, in the air (where they are carried about on particles of dust), and in food and drink, and, moreover, great numbers of them live constantly on the skin and other body surfaces, and in the intestinal canal of healthy men and animals.

Obviously, life as we know it, could hardly be possible if *all* of these micro-organisms, so widely distributed, were capable of causing disease. As a matter of fact, the disease-producing microbes are decidedly in the minority. The one place in which microbes are *not* naturally to be found is within the healthy tissues of the living body. Among the great host of microscopic creatures there are only about fifty common varieties that are able to grow in, or otherwise damage, the living tissues of human beings. The great majority of microbes perform functions which are useful to man, and some, as we shall describe, play an indispensable rôle in the economy of nature.

Some of the microbes now existing, such as certain bacteria found in the soil and in water, appear to represent extremely simple and primitive forms of life. They seem to have retained the characteristics of those earliest forms of microbic life that must have been abundant on the earth long before higher plants, animals, and human beings were evolved. Other present day species, however, show extraordinary adaptations to a particular mode of life in association with particular living hosts, which must be the result of a long evolutionary development.

Saprophytic and parasitic microbes. Among all these many kinds of micro-organisms, two distinct groups may be recognized on the basis of their distribution in nature and the character of their food requirements: (1) *saprophytes,* and (2) *parasites.*

Saprophytes ("rot-plants") are so called because the most conspicuous result of their growth is the rotting or decomposition of organic matter. Organisms of this class are very widely distributed in the soil, in natural waters, and elsewhere throughout nature and *their food is nonliving matter.*

Parasites are adapted to living on or in the living bodies of plants, animals, or human beings. Their most conspicuous property is their dependence upon other living things; *their food is derived directly from living matter.*

Pathogenic and nonpathogenic microbes. Forms of parasitism. It is important to remember that *almost all the saprophy-*

tic organisms and by far the great majority of parasitic microbes are entirely harmless (*nonpathogenic*) and have nothing to do with the production of disease.

Many of the bacteria found constantly inhabiting the skin or other body surfaces are truly parasitic, for they are dependent for existence upon their relationship with their living host, yet they are never responsible for disease. These harmless parasites are usually referred to as *commensals,* a word meaning "those who partake of the same food, or eat at the same table." *Commensalism* is an association in which the micro-organism, i.e., the commensal, derives benefit, but does not injure or benefit the host.

Another less common form of parasitism is called *symbiosis,* a "living together." In this type of association both partners are benefited, and often the very existence of one or both depends upon their symbiotic relationship. For example, certain bacteria in the nodules on the roots of leguminous plants, such as clover, take up nitrogen from the air, and part of this nitrogen then becomes available for use by the plant, while at the same time the bacteria are nourished from the roots.

In general it is true that most of the pathogenic organisms, especially those causing communicable diseases, are adapted to a rather strict parasitic life. Most of the pathogenic microbes are parasitic organisms which can live not only upon the *surface* of the body, as the nonpathogenic parasites do, but can multiply *within the tissues* of the body.

Infection. When micro-organisms enter the body tissues and cause injury, we say an *infection* has occurred; the individual is *infected,* and suffers from an *infectious disease.*

Most of the infectious diseases are rightly regarded as examples of a special form of parasitism.

REVIEW QUESTIONS—CHAPTER IV

1. What is a cell? How did the term originate?
2. What is meant by (*a*) multicellular organisms, (*b*) unicellular organisms? Give examples.
3. What is protoplasm? Describe its general character.
4. Describe the structure of a typical plant or animal cell. Define *nucleus, cytoplasm.*
5. Compare bacterial cells with the cells of higher plants and animals.

6. List five vital functions of living cells. Explain each briefly. Do single-celled micro-organisms possess these properties?
7. What are the main groups and subgroups into which living organisms are classified?
8. What constitutes the scientific name of an organism? How is it properly written?
9. Name the seven principal groups of micro-organisms.
10. What unit of measurement is used to express the size of microbes? Compare the different types of microbes as to size.
11. What are the *Protozoa?* Give examples of protozoa belonging to each of the four classes.
12. Why are all the microbes, except the protozoa, classed in the plant kingdom? What are the two great classes of plant-like micro-organisms?
13. Show by a diagram how the bacteria and true fungi fit into the plant kingdom.
14. Describe briefly the *Thallophyta, Algae, Lichens.* What kinds of organisms are included in the great group of plants called *Fungi?*
15. What are the *Eumycetes?* Define *mycosis, mycotic disease.*
16. Give examples of organisms belonging to the different classes of true fungi.
17. Give the scientific name for bacteria, and its literal meaning.
18. In common usage, what is meant by the term "*fungi*"?
19. Name the three principal orders of the *Schizomycetes.*
20. Describe the spirochetes and name an important pathogenic spirochete.
21. Name and describe briefly the species of mold-like higher bacteria which causes actinomycosis.
22. Name two other important disease germs which are usually classified as higher bacteria.
23. What is the scientific name for the true bacteria? Name and describe briefly the principal families. How do these organisms compare in size and form with other kinds of microbes?
24. What can be said about the classification of the rickettsiae and the filtrable viruses? How large are rickettsiae?
25. Discuss the distribution of micro-organisms and their place in nature.
26. Define *saprophytic, parasitic, pathogenic, nonpathogenic microbes.* What is meant by *commensalism, symbiosis, infection?* Give examples to illustrate the meaning of all these terms. In general what proportion of microbes are harmful?

CHAPTER V

THE PROTOZOA

THE *PROTOZOA* are unicellular micro-organisms, the lowest forms of life classed in the animal kingdom. Their single-celled form distinguishes them from the *Metazoa*—the larger, multi-cellular animals.

Free-living and parasitic protozoa. Two great groups among the protozoa may be recognized: (1) the *free-living* forms, and (2) the *parasitic* forms.

Free-living protozoa are to be found in fresh or salt water, in the soil, and in decaying vegetable matter everywhere. Parasitic protozoa occur in association with practically every kind of living animal, and there are even some that parasitize other protozoa. These parasitic forms are naturally more limited in distribution than the free-living organisms, occurring only in the particular human or animal hosts, or small group of related hosts, to which they have become adapted. The majority of these microbes exist as harmless *commensals*. Protozoa of this type are commonly found, for example, in the intestinal contents of man and animals.

Classes of protozoa. As we have previously indicated, there are four main classes of protozoa. These are:

(1) **Sarcodina** (= "flesh-like" organisms), the most important subgroup of which is the **Amebae.** These exist as naked globules of protoplasm, and are characterized by the use of *pseudopodia* as organs of locomotion and for the capture of food.

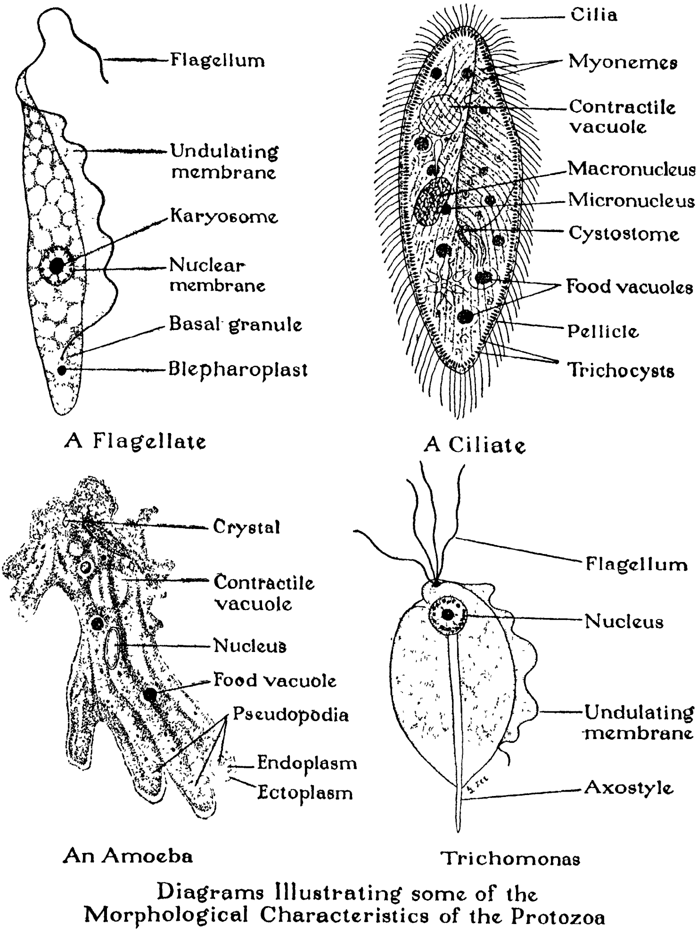
(2) **Flagellates,** also called the **Mastigophora** (= "whip-bearing" forms). These swim about by means of long whip-like projections from the cytoplasm, called *flagella.*

(3) **Infusoria,** also called the **Ciliates.** These have the most elaborate internal structure of all protozoa. They move about actively, propelled by the sweeping movement of innumerable, short and delicate, hair-like processes, called *cilia,* on their surface.

(4) **Sporozoa.** These protozoa have no motor organs. They

are relatively small, and they all live as *parasites* in the cells or tissues of other animals. At some stage in their life cycle they reproduce by the formation of multiple *spores.*

Most protozoa are readily distinguished from all other types of micro-organisms. They differ from bacteria in several respects:



A Flagellate

A Ciliate

An Amoeba

Trichomonas

FIG. 12. Morphological features of the Protozoa. (A ciliate, redrawn and modified from Wenvon: other figures, redrawn and modified from Kudo)

(1) they are usually larger, (2) they are nucleated cells with a more complicated internal structure, and (3) they reproduce in various complex ways.

Morphology and physiology. Some of the outstanding morphological features of the protozoa are illustrated in Fig. 12. Among these creatures are to be found the largest and most complicated of all single-celled organisms. Some species parasitic in man have a diameter of 30 to 50 micra, and certain varieties living free in nature reach a length of 600 micra or more. Although the whole organism is a single cell, many protozoa have a most complex internal structure, including an arrangement of organelles (for the capture, digestion and storage of food, for the excretion of liquid and solid wastes, for protection and support of the body, and for reproduction) which function like the organs of higher animals (Figs. 11, 12, 13).

The life processes of protozoa are carried out in more specialized ways than is the case with the simpler bacteria and fungi. The great majority of protozoa, both free-living and parasitic, are distinctly animal-like in their nutritional habits, since they take into their bodies, often through a specialized mouth, solid food particles, digest these and then cast out the indigestible residue. When the common *Paramecium* is grown in a laboratory culture together with bacteria, it does not derive its food from the medium, but feeds upon the bacteria themselves. There are some exceptions to this general rule, however, notably the parasitic protozoa of the class *Sporozoa*, which are nourished only by direct absorption of soluble food materials through their cell walls.

Many species of protozoa, including pathogenic varieties, may be grown in test tubes in the laboratory on media containing blood and other complex protein substances, similar to the concoctions used for the cultivation of pathogenic bacteria.

Encystment. The wide occurrence of free-living protozoa is due in part to their ability to *encyst*, that is, to change into resting forms, called *cysts*, which are resistant to drying and other unfavorable conditions. These cysts are carried about in dust, on insects, and in other similar ways, and are always ready to germinate into active, growing protozoa whenever they happen to reach a suitable environment.

Most of the parasitic protozoa also may encyst when their immediate environment is drying up or otherwise becoming unfavorable. Cysts of the intestinal protozoa form in the lower intestine and are evacuated in the feces. Fecal material thus becomes the source of infection for new hosts.

Reproduction. *Asexual reproduction.* A simple form of multiplication, seen, for example, in the paramecia and other ciliates, and in amoebae, consists of a simple fission of the nuclei followed by a splitting of the cytoplasm of the protozoan across its short axis into two approximately equal parts. In another form of the reproductive process, common in flagellates, the nucleus first undergoes more or less complicated changes similar to the mitosis which regularly occurs when cells of higher plants and animals multiply, then the organism splits into two parts by *longitudinal* division of the cytoplasm.

In *Sporozoa* the characteristic method of reproduction is by *multiple division* (sporulation). The mature protozoan becomes divided throughout into a number of daughter cells, each with its own nucleus, thus forming a mass of new cells, which finally separate and develop individually. When this process of multiple division is entirely asexual, and occurs without preliminary formation of a cyst, it is called *schizogony*, and the new cells are called *merozoites.* A similar process occurring within a cyst, and (usually) after the encysted female cell has been fertilized by previous union with a male cell, is called *sporogony*, and the new cells are called *sporozoites* (Fig. 100).

Sexual reproduction; life cycles. At some time in the development of practically all varieties of protozoa the asexual processes of multiplication mentioned above are interrupted, and sexual phenomena intervene. Sexual activities among the protozoa involve the coming together of individuals of the same species in such a manner that nuclear material is exchanged or fused.

When this union is temporary it is called *conjugation.* The individuals concerned, after attaching themselves to each other for a time, during which nuclear matter is exchanged, separate completely and each then continues to grow and multiply independently.

When the union is permanent, and the substance of the two cells becomes fused, this is called *copulation.* The individuals taking part here are sometimes morphologically alike, but more commonly they differ in size and form in a manner comparable to the spermatazoon and ovum of the multicellular animals. In the highest development of this sexual type of multiplication the entire process is amazingly similar to reproduction among higher animals.

The series of changes a protozoan undergoes from one act of fertilization to another constitutes the *life cycle* of that organism. Many parasitic protozoa pass through most complicated life cycles, during which they may assume quite different forms, and multiply in different ways. Many pathogenic species pass the sexual part of their cycle exclusively in one host, and the asexual part in another host. For example, the malaria parasites develop, in the sexual phase of their cycle, only in the body of the malaria-carrying *Anopheles* mosquitoes, where they multiply by sporogony, while within the red blood cells of their human hosts, they multiply only asexually, by schizogony (Fig. 100).

Amebae. These are the most important protozoa of the class *Sarcodina.*

The most characteristic feature of the amebae is their curious method of locomotion. In response to a physical or chemical stimulus, they send out irregular extensions of their protoplasm, called *pseudopodia* (false feet). Then the rest of the cell *flows* along after these extensions.

This flowing motion is seen not only in the amebae, but in certain cells which make up the bodies of higher plants and animals. For example, the white blood cells (leukocytes) of the human body, are able to move about in this way. This kind of movement is so characteristic of the amebae that, wherever observed, it is referred to as *ameboid motion.*

Harmless, free-living amebae are widely distributed in nature. An example is the *Ameba proteus* (Figs. 12, 13: A). It appears as an irregularly shaped globule of protoplasm, containing a nucleus, a contractile vacuole, food vacuoles, and other inclusions. This organism and related free-living species are relatively large, often reaching a diameter of 600 micra or more.

Parasitic amebae are to be found regularly in association with practically every type of living animal, and also in man. The most important nonpathogenic amebae present in human beings are called *Endameba gingivalis* and *Endameba coli.* The former species is found occasionally in the tartar about the teeth in the human mouth. It is a fairly active ameba, averaging about

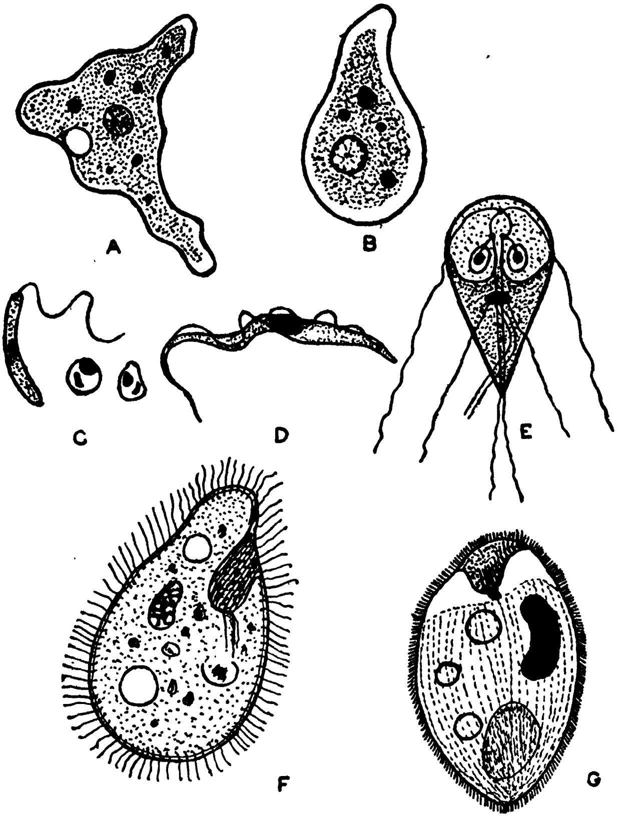


FIG. 13. Various protozoa. A, a harmless ameba. B, *Endameba histolytica* the cause of amebic dysentery. C, forms of *Leishmania,* a kind of protozoan causing sores in the skin. D, a *trypanosome,* a flagellated protozoan of the type causing African sleeping sickness. E, *Giardia lamblia,* a common intestinal parasite of man. F, a *paramecium,* a harmless ciliated protozoan common in nature. G, *Balantidium coli,* a ciliate sometimes found in the intestine of man. (Figures D, E, and G redrawn from Hegner, Cort, and Root.)

15 micra in diameter. It is entirely harmless. *Endameba coli* is a common commensal in the human intestine. The active forms vary from 15 to 40 micra in diameter. Cysts are passed in the feces and reach new human hosts through contaminated food or drink. This harmless ameba is important medically

because it may be confused with the variety causing amebic dysentery.

The only ameba pathogenic for man is the *Endameba histolytica*, the cause of amebic dysentery and other forms of amebiasis (Figs. 11, 99). This disease is so important that we give it special consideration in Chapter XLI.

Flagellates (Mastigophora). Protozoa of this class move by means of the delicate processes called *flagella* which propel the organisms along by their whip-like motion. In addition to flagella, some flagellates possess an *undulating membrane.* There are numerous distinct varieties, showing great differences in size and details of structure.

Included among the free-living forms are species like *Uroglenopsis americana* and *Volvox globator,* that sometimes grow so abundantly in the water of reservoirs that the oily secretions from their bodies add a disagreeable odor to the water.

Parasitic flagellates include several species that are found as commensals in the human digestive tract, mouth, or vagina. The best known of these belong to the genera *Trichomonas* and *Giardia.*

Trichomonas hominis and *Trichomonas vaginalis* are sometimes present in the intestine and in the vagina, respectively. They are pear-shaped organisms about 10 to 20 micra long (Fig. 12). Probably neither of these microbes is pathogenic by itself, but the latter species becomes very abundant in certain cases of acute vaginitis, and may be in large part responsible for the inflammation. *Giardia lamblia* is found most often in the feces of individuals who have diarrhea, but the organism is probably not able by itself to cause disease and ordinarily exists harmlessly in the intestine. The peculiar, bilaterally symmetrical, pear-shaped body, gives this protozoan a most striking appearance (Fig. 13: E).

The most important pathogenic flagellates are the *Trypanosomes* and the *Leishmania.*

Trypanosomes of many different species are found as parasites in the blood stream of horses, cattle and other vertebrate animals, and in man. Typical trypanosomes have a narrow, elongated, leaf-like body, pointed at the anterior end and more or less blunted at the opposite pole. The flagellum arises from the blepharoplast, or a basal granule nearby, at the blunted end, and passes along the body, marking the outer margin of a well developed undulating membrane, and extending outside the body at its anterior end (Figs. 12, 13: D). There is a single large nucleus. Reproduction is entirely asexual, usually by longitudinal division. At certain phases of their development trypanosomes may assume smaller and less complex forms. These parasites are carried from host to host by blood-sucking insects or arthropods. It is probable that they were originally insect parasites, which have become more or less fully adapted to the blood of vertebrate hosts.

Trypanosoma gambiense and *Trypanosoma rhodesiense* are responsible for *African sleeping sickness*, a severe and usually fatal disease of tropical Africa. (This is an entirely different disease than the so-called "sleeping sickness"—lethargic encephalitis—which occurs in this country.) The African disease is carried from person to person through the bite of the blood-sucking *tsetse fly*.

Trypanosoma cruzi is the cause of "Chagas's disease," another form of trypanosomiasis occurring in South America. This is also transmitted by biting insects.

Leishmania occur as minute, rounded or oval bodies, about 2–5 micra in diameter (Fig. 13: C). The clear cytoplasm is surrounded by a delicate membrane, and contains a nucleus and a smaller, oval or rod-shaped body, from which a flagellum arises when the organism enters the motile phase of its development. In the infected vertebrate host the nonmotile parasites invade and multiply within certain body cells of the spleen and other organs, and also a few may develop within some of the white blood cells of the circulating blood. The parasites are transmitted by various types of blood-sucking insects, in which they grow as flagellated forms.

Leishmania donovani is the cause of the tropical disease in man called kala-azar. *Leishmania tropica* is the species found infecting the skin, mouth, or nose and causing the condition known as "Oriental sore." *Leishmania braziliense* is responsible for a disease occurring in Brazil and other parts of South America, characterized by ulcerations of the mucous membranes.

Infusoria. This class of protozoa contains a great number of free-living species, and some that are parasitic on fish and various animals. They are all characterized by possession of *cilia* which serve for locomotion and the capture of food. Among these ciliated organisms are found the protozoa with the most elaborate differentiation of structure. Conjugation is the usual form of sexual reproduction. Asexual multiplication occurs through transverse fission.

A typical and well known example of a free-living ciliate is *Paramecium caudatum* (Figs. 12, 13: F). This and related species are large slipper-shaped, or cigar-shaped cells, with a wide and conspicuous cystostome (mouth). They are commonly about 200 micra in length.

Balantidium coli (Fig. 13: G) is the only species of medical interest in the whole class of *Infusoria.* This organism is the largest of the human intestinal protozoa, often measuring 50--100 micra long and 30–70 micra broad. Large round cysts, 60 micra in diameter, are formed. The organism is a common intestinal parasite of pigs, and human beings are presumably infected by ingestion of cysts excreted by these animals. It may cause a form of chronic dysentery in man.

Sporozoa. All the members of this class are relatively small, nonmotile organisms, adapted to an exclusively parasitic life. Many have complicated life cycles, involving existence in two quite different hosts in succession. They all form spores (sporozoites) at some time in their development. Many multiply by schizogony (asexual multiple division) during part of their life cycle, and by sporogony, following copulation, in the sexual phase of their development.

In practically every type of animal, parasites of the class *Sporozoa* may be found, living in the blood cells, or other tissues, or in body cavities. Some produce no evident illness in their hosts, while others are responsible for some of the most severe and widespread of protozoan diseases. (Fig. 11:4)

By far the most important pathogenic varieties are the parasites of human malaria. (Similar organisms infect monkeys and birds.) These are classed in the order *Hemosporidia* and in the genus *Plasmodium.* The three principal species that cause human malaria are *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium falciparum.* They undergo an asexual development in the red blood cells of their human hosts, and a sexual transformation in the digestive tract of mosquitoes of the genus *Anopheles.* Malaria is discussed in Chapter XLI.

REVIEW QUESTIONS—CHAPTER V

1. Distinguish between protozoa and metazoa.
2. What two great groups among the protozoa may be recognized, and how are these distributed?
3. Name and describe briefly the four main classes of protozoa.
4. In general terms, how do protozoa differ from bacteria?
5. Describe the form and structure of some typical protozoa. Discuss the nutritional habits of protozoa. Can they be cultivated artificially?
6. What is meant by encystment of protozoa? Explain its importance in making possible wide distribution of free-living and parasitic species.
7. Describe forms of asexual reproduction among the protozoa. Define *multiple division*, *schizogony*, *merozoites*, *sporogony*, *sporozoites.*
8. What is meant by *conjugation*, *copulation*, in protozoa?
9. What is the "life cycle" of a protozoan? What common pathogenic protozoa have a complicated cycle?
10. Describe locomotion of an ameba; define *pseudopodia*, *ameboid motion.*
11. Describe a common, free-living ameba.
12. Name and describe a species of parasitic ameba found (1) in the human mouth, (2) in the normal intestine.
13. Name the ameba responsible for amebic dysentery in man.
14. Define *flagella*, *undulating membrane.*
15. Name and describe flagellates commonly found in the (1) human digestive tract, (2) vagina.
16. Describe *Trypanosomes* and *Leishmania.* Name three pathogenic species of trypanosomes and three of leishmania, and discuss briefly the diseases they cause.
17. What properties characterize the *Infusoria?* Name and describe a common free-living ciliate.
18. Describe a pathogenic ciliate.
19. What properties characterize the *Sporozoa?* Where are these protozoa found?
20. Name the most important species of *Sporozoa* pathogenic for man. What hosts serve for the (1) asexual and (2) sexual phase in the life cycle of malarial parasites?

CHAPTER VI

THE FUNGI AND THE MOLD-LIKE HIGHER BACTERIA

THE TRUE FUNGI

THE "TRUE fungi" comprise the order of microbes called the *Eumycetes* (Fig. 11: 5, 6, 7). They form a large and heterogenous group of primitive organisms, all lacking the chlorophyll always associated with the familiar green plants, but having, nevertheless, a structure and mode of life definitely plant-like. Included are the microbes generally called the *yeasts* and the *molds,* as well as another large group known as the *fungi imperfecti.*

Fungi of many different kinds are widely disseminated. The majority are saprophytic, and only a few are adapted to a fully parasitic existence. Even so, fungous infections of higher plants and animals are common, and in human beings diseases caused by fungi (*mycoses* or *mycotic infections*) are among the most troublesome of all afflictions. On the other hand, among the saprophytic fungi are some of the most genuinely useful microbes known to man, such as the bread and wine yeasts, and others used in industrial processes.

Molds and yeasts. In their systematic classification of the fungi, mycologists do not recognize "molds" as a distinct botanical group. But everyone knows what is meant by a *mold,* in the popular sense of the term. Common molds are the most familiar of all the microbes to the layman. Who has not seen moldy food, moldy grain? To the naked eye molds may appear like a tuft of cotton wool, or a sooty black patch, or a fuzzy or powdery mass, variously colored. Most often we find them growing on old moist bread, jellies, fruits, or other foods, or appearing as contaminants in laboratory culture media. There is a characteristic "moldy" smell. Under the microscope the growth is seen to be made up of a network of branched threads, called the mycelium, and, usually, there are small round bodies present also, which are the reproductive elements or *spores*. This filamentous, web-like structure, and the abundant spores, are the outstanding characteristics of these micro-organisms (Fig. 15).

In contrast to the somewhat complicated makeup of these familiar molds, the common *yeasts*, such as those in the ordinary yeast cake, are fungi of much simpler form, for each individual organism exists as a single, independent, microscopic, round or oval cell. Unlike the molds, the yeasts never develop branched filaments, and they characteristically multiply, not by free spores, but by budding (Fig. 11:5).

Because of these clearcut differences in structure and in mode of reproduction everyone finds it convenient and useful to recognize the yeasts as one group of fungi, and the common filamentous molds as another distinct group. It must be realized, however, that this customary grouping of the fungi into yeasts and molds cuts across the limits of strict botanical classification. A "mold" is not a scientific term that can be precisely defined, but is loosely used to mean any kind of filamentous fungus. Again, mycologists regard yeasts not as an independent class of micro-organisms, but as a group of fungi of heterogeneous origin, characterized by permanent loss of the power to grow as branched filaments, so that they exist now only as separate, budding cells. And from the botanical point of view, as we have indicated in the diagram on page 45, the common yeasts belong to the very same class of fungi (*Ascomycetes*) as some of the most familiar and typical of the molds. Even so, we shall continue to call *molds* all those fungi, regardless of their strict classification, that grow in the form of branched filaments (thus making a mycelium), and to call *yeasts* those single-celled fungi that grow independently without ever developing a mycelium.

Classes of fungi. As we have seen (p. 45), the *Eumycetes* are divided into 4 classes: (1) *Phycomycetes*, (2) *Basidiomycetes*, (3) *Ascomycetes*, and (4) *Fungi imperfecti*. The last group is commonly called *Hyphomycetes* in medical writings.

This classification is based, first, upon differences in the structure of the filaments, when present. An individual filament is called a *hypha*, and the mass of branched hyphae that makes up the bulk of a filamentous fungus constitutes the *mycelium*. In most fungi the mycelium is *septate*, that is, the hyphae are divided by cross-walls (*septa*) into a series of cylindrical cells, end to end. But in one class (*Phycomycetes*) the filaments in the growing organisms are *nonseptate.* Secondly, classification is based on the manner of reproduction, especially the way in which sexual spores, if any, are formed. The following scheme shows the principal differential characteristics. The meaning of the terms used will be more fully explained below.

Eumycetes
(true fungi)

Nonseptate mycelium
1. **Phycomycetes**—asexual spores (sporangiospores) formed in a closed structure (sporangium); produce zygospores (a kind of sexual spore).

Septate mycelium (when present)
2. **Basidiomycetes**—sexual spores borne on special club-like stalks (basidia)
3. **Ascomycetes**—reproduce most commonly by asexual spores (conidia), but all are capable of forming sexual spores at times (ascospores) in an especially developed little sac (ascus)
4. **Fungi imperfecti**—(*Hyphomycetes*)—no sexual spores; reproduce by various types of asexual spores, including blastospores (budding).

Phycomycetes. Included in this class are the common water molds that sometimes appear in goldfish aquaria, and related forms that parasitize water plants and fish. These are primitive fungi, apparently closely related to certain varieties of protozoa and algae. *Phycomycetes* means literally "seaweed fungi." Also in this class belong some of the most widely distributed and best known of the common molds—those white, cottony growths so often seen on barnyard manure, decaying foodstuffs, and as contaminants in Petri dish cultures. These molds are of the genus *Mucor*, or *Rhizopus.*

Basidiomycetes. Here belong the large, fleshy fungi, such as the toadstools, puff-balls, and mushrooms, and also smaller forms which are important plant parasites, known as smuts and rusts. Many of the latter types have complicated life cycles, involving a sexual phase in one plant host, and asexual phases in a different plant host, recalling the similar cycles of parasitic protozoa. None of these microscopic fungi are pathogenic for man or animals.

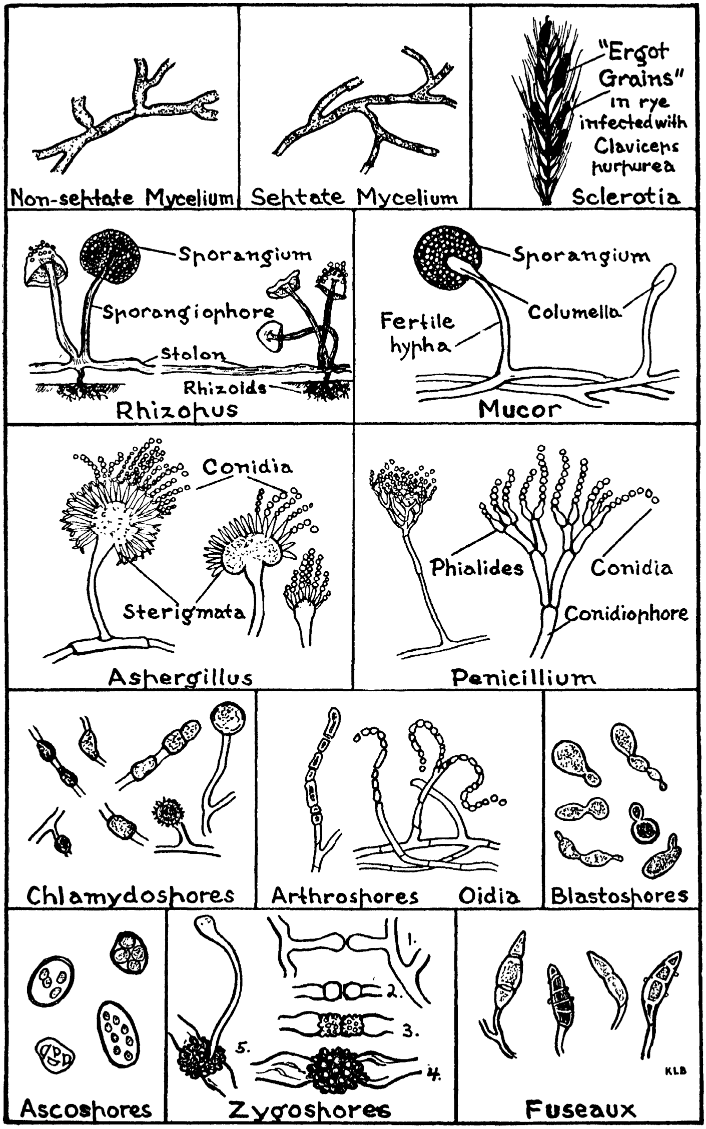


FIG. 14. Some of the morphological features of the fungi.

Ascomycetes. This is the largest class of fungi, containing numerous important species. The many varieties included here differ materially from each other, yet have one feature in common—namely, they all form, after a kind of fertilization process, sexual spores called *ascospores.* These are developed within an especially evolved sac, known as an *ascus.*

Common possession of this one structure brings together into this botanical class fungi which represent the opposite extremes of complexity—the typical, single-celled, true yeasts (genus *Saccharomyces*) on the one hand, and the common, highly organized, multicellular molds (such as those of the genus *Penicillium* and *Aspergillus*) on the other.

Fungi imperfecti, or Hyphomycetes. In this large class are placed those fungi which do not form ascospores, or other types of sexual spores—or at least, such spores have not been observed. Hence these organisms have an imperfect—or imperfectly known—life history.

The study of these "imperfect" fungi is difficult. We encounter among them bewildering irregularities in form and development; many have an abundant mycelium at times, while at other times they grow as yeast-like cells, and reproduction occurs through spores elaborated in a confusing variety of ways. This creates practical difficulties in the diagnosis and study of fungous infections, for we find the *majority of fungi pathogenic for man and animals* among the *Fungi imperfecti.* We must utilize a working classification of the pathogenic fungi based only in part on botanical considerations (See below).

Morphological features of the fungi. The structure of various types of fungi is illustrated in Fig. 14, and also in Figs. 11, 15 and 16. These drawings, and the accompanying descriptions, should be consulted while the organisms themselves are being studied in the laboratory, and while reading the paragraphs that follow. The methods used in the microscopical examination and cultivation of the fungi are described in Chapters XII and XIII.

THE COMMON MOLDS

The molds which most commonly appear upon food and in agar cultures which have been opened to the air belong to one of four genera: (1) *Rhizopus,* or (2) *Mucor,* (of the class *Phyco-*

mycetes), and (3) *Aspergillus,* or (4) *Penicillium* (of the class *Ascomycetes*).

Rhizopus nigricans is perhaps the most frequently seen of all the molds. It is often found on moldy bread, and as an air contaminant in Petri dish cultures in the laboratory. When the spores settle upon an agar plate they soon germinate and a meshwork of white filaments develops, which may fill the entire dish. This mold grows rapidly over the whole surface of the medium by means of its stolons—branches which extend away from the main body of the growing mold, like the runners of a strawberry plant. Where the tips of the stolons come in contact with the medium a group of short, root-like submersed hyphae (rhizoids) develops. Often the stolons climb the sides of the Petri dish and run along the underside of the lid, the rhizoids attaching themselves to the glass. At the points in the runners where the rhizoids appear, fertile aerial hyphae rise, and eventually bear the black spore-cases (sporangia) within which the asexual reproductive spores (sporangiospores) are formed.

Mucor mucedo is the best known of the many species of the genus *Mucor.* It can always be obtained by incubating fresh horse manure in a moist chamber. Its spores are common in dusty air. Like *Rhizopus* it forms a luxuriant, and rather coarse, nonseptate, white mycelium and black sporangia, and it may likewise fill a Petri dish in twenty-four hours. There are no stolons, or rhizoids, however, and the mold does not attach itself to the lid of the dish. The fertile hyphae arise from all parts of the mold, and to the naked eye the sporangia appear as black dots scattered uniformly throughout the mycelium. On microscopic examination one can see that the sporangium of *Mucor* is built about a cylindrical columella, whereas in *Rhizopus* the structures are somewhat more complicated (Fig. 14).

In both *Mucor* and *Rhizopus* molds sexual spores (zygospores) are occasionally formed by the union of two hyphae arising from neighboring growths. The zygospores are usually large, black structures covered with a rough, warty membrane (Fig. 14).

Aspergillus niger is the most common of the many varieties of this genus. It grows preferably on materials rich in sugar and of an acid reaction, but may be found on a great variety of substances. Some colonies will usually appear on agar media exposed for a time to dusty air. A white, or gray, septate mycelium forms, and soon the mold becomes recognizable by its characteristic fruiting bodies. The fertile hyphae are enlarged at their ends into a rounded body, from which small stalks (sterigmata) radiate. These primary sterigmata branch, in this species, to make still smaller projections, and on the tips of these are borne chains of black, or brown spores (conidia). The chains of conidia are so closely packed that the supporting structures often are hidden from view, and we see only the large, black spore-heads made up of a globular mass of pigmented spores. These "heads" are easily visible to the naked eye (Fig. 11:6).

Other varieties of *Aspergillus* have spores of other colors, such as green or yellow, and also the mycelium may be variously colored. These microbes generally prefer a somewhat higher temperature than other common molds, and many grow especially well at body temperature (37° C.). They are often found on the human body, and at least one species, *Aspergillus fumigatus*, is pathogenic.

Penicillium glaucum is the name commonly applied to the familiar blue-green molds. These are often seen on fruits, and also may contaminate laboratory cultures, though not quite so frequently as the other molds already mentioned. They are readily distinguished by the peculiar structure of their sporophores. The fertile hyphae branch toward their ends and finally terminate in a radiating cluster of flask-shaped cells (phialides) from the tips of which the small, round conidia develop. These spores are formed by successive divisions of the terminal cell and are pushed outward, so that the oldest spore is at the outermost end of the chain. The whole spore-head under low power magnification looks somewhat like a tassel, or brush, and this is the reason for the name *Penicillium*, derived from the Latin *penicillus*, a brush. The mass of newly-formed spores gives the mold a powdery appearance to the naked eye.

There are numerous varieties of *Penicillium;* many have distinctive colors because of pigments contained in the conidia. Two species of special interest are *Penicillium roqueforti*, which is grown in Roquefort cheese to give it its characteristic flavor, and *Penicillium camemberti*, the mold in Camembert cheese.

Importance of the common molds. When mature, the spores of the common molds are swept off by wind currents into the air, and thus they become scattered everywhere. These spores are resistant to cold, drying, and moderate heat, and may remain alive for long periods. They are always present in the air of rooms, especially dusty rooms, and even in the refrigerator. They are always ready, when they fall on food, or other substances which supply moisture and the small amount of organic matter they need for growth, to develop into new molds.

Some types of molds will grow on almost any object, including the walls and floors of a building, in the absence of sunlight and in the presence of moisture. In localities where the atmosphere is warm and humid, molds may develop on soiled, damp towels or clothing in the laundry bag, on the old shoes stored away in the closet, and on the books on the library shelves.

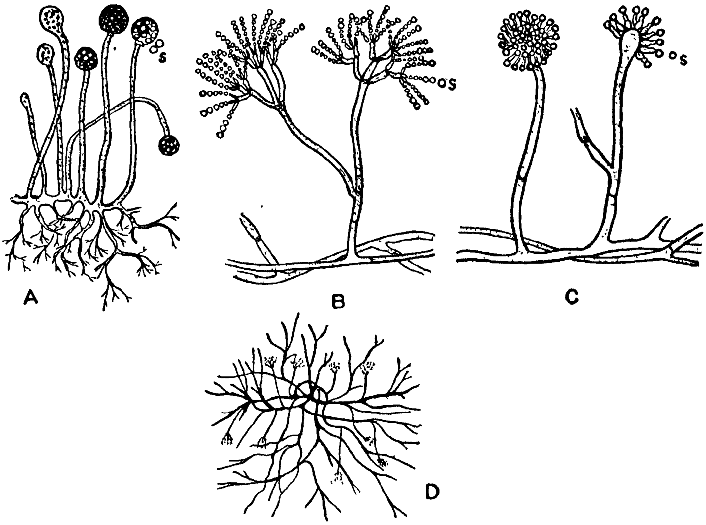


FIG. 15. Diagrams of common molds. A, *Mucor;* B, *Penicillium;* C, *Aspergillus.* Figures show the characteristic fruiting bodies in which the reproductive spores (S) are formed. D, a sketch to illustrate the network of branched threads (mycelium) of which a mold is composed.

Molds sometimes grow extensively in the humid rooms of bakeries, and in creameries they may contaminate the butter churns. Certain kinds cause deterioration of textiles and the dry-rot of lumber. Aspergilli and other common varieties cause much economic loss when they grow upon strawberries and other fresh fruits as these are being shipped to market. Foods which are well preserved from decomposition by *bacteria,* may nevertheless be spoiled by the growth of molds, because the latter develop (though slowly) at low temperatures, in materials with a high concentration of sugar or salt, and in substances that are strongly acid. So we find that such foods as preserved fruit and jellies, butter, pickles, sauerkraut, vinegar, and salted and smoked meats may become moldy, even though kept in a cool place. The housewife often finds that some of her jars of home canned foods, not having been sealed perfectly, in time show a growth of molds.

In the home and the hospital, the growth of molds is reduced by general cleanliness and by removal of dust. It is well to remember that dry objects do not "mold" easily and sunlight and cold keep molds down. Home canned foods must be thoroughly heated when canned and the container must be hermetically sealed from the air. There is probably no danger in eating foods which have become contaminated with the ordinary molds. The acute intestinal disease, popularly called "ptomain poisoning," is *not* caused by moldy food.

All of the common types of molds are occasionally found in inflamed ears, eyes, nose, or skin of human beings, though there is usually no clear evidence that they are in any degree responsible for the inflammation. The *Aspergilli* have been observed especially often in lesions of the lungs and the species *Aspergillus fumigatus* is definitely pathogenic. Molds are the cause of some cases of *asthma* in individuals who are hypersensitive to the spores when these are inhaled.

Molds are helpful in some circumstances. Those in the soil contribute, along with the bacteria present, to the breakdown of complex organic waste into simpler nitrogenous compounds which may be utilized by plants for food. Certain varieties are widely used in the Orient for the conversion of rich starch to sugar as the first step in making alcoholic beverages. The preparation of citric acid from cane sugar by action of molds is an example of the commercial use of these organisms. We have already mentioned that some species of *Penicillium* are responsible for the characteristic flavor of certain popular types of cheese.

THE YEASTS

The yeasts make up a group of fungi whose activities have always been of great practical importance in human affairs. Their usefulness in the preparation of bread, beers and wines is well known to all. These microbes are widespread in nature; they are especially abundant wherever much sugar is present. Some varieties are always to be found, for example, in foodstuffs, especially cream and other dairy products, in honey, the nectar of flowers, the exuded sap of trees, and upon ripe fruits, notably grapes. They occur also in soil, on vegetation, and as commensals in insects and animals.

Chief characteristics. The yeasts stand out from all the other fungi because they occur constantly as single, budding cells, and do not form the branching filaments (mycelium) which characterize the molds. Many other fungi *temporarily* assume a single-celled, budding form, but eventually develop a mycelium under proper conditions. Only the genuine yeasts never do. The yeasts have almost certainly originated from molds by losing permanently the power to form a mycelium (Fig. 16).

The typical yeast cell is a colorless, oval, or round body, averaging about 10–15 microns in diameter. The size varies greatly. Many yeasts have thick walls, giving the cells a double contour or outline. Each organism has a nucleus (though this is not often visible). The cytoplasm, especially in older individuals, commonly contains many granules and vacuoles.

A few kinds of yeasts multiply by splitting of the cell into two parts (fission) like the bacteria, but all the common varieties reproduce by *budding*. In the budding process a small swelling first appears at the edge of the cell. The swelling gradually increases in size and becomes constricted at the base, so that it has a very narrow connection with the parent cell. This knoblike protrusion is the bud, or blastospore. The bud finally becomes entirely separated from the parent organism and develops into a full sized yeast. Then it, too, may form buds and so continue the reproductive process. When growth is rapid a series of buds may appear, which do not fully separate, forming clusters or short chains of oval units.

Budding does not occur among the true bacteria. The yeasts are therefore distinguishable from bacteria on this account. Yeasts differ from bacteria also in the following ways: they are much larger, and the cells are nucleated and usually have a double-contoured wall.

Two main groups are recognized among the yeasts: (1) the common, harmless bread, beer, and wine yeasts (mostly placed in the genus *Saccharomyces*), which occasionally form *ascopores* in addition to reproducing by budding, and (2) a group of yeasts named the *Torulae*, that multiply by budding but do *not* form spores. Among the *Torulae* there are both harmless and highly pathogenic species.

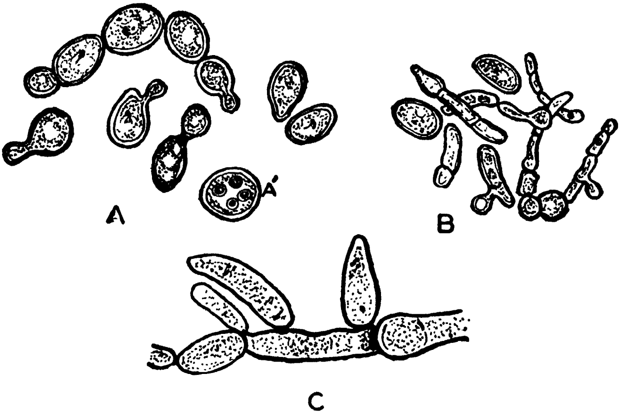


FIG. 16. Yeasts. A, Common harmless yeast (*Saccharomyces*), showing budding. A′, formation of ascospores, a characteristic property of true yeasts. B, *Blastomyces*, a pathogenic yeast. C, *Monilia*, a yeast *like* fungus causing thrush. (Figure C drawn from a photograph in Zinsser.)

Saccharomyces. These kinds of yeasts have been extensively studied because of their great importance in the manufacture of beers, wines, and bakery goods, and their value in other industries which depend upon a process of fermentation (decomposition of sugars and similar substances). The relation of the yeasts to fermentation was discovered as early as 1837 by Schwann, but the complete demonstration of the fermenting powers of yeasts was first given by Pasteur about 1865. The studies of the Danish investigator Hansen about 1880 added much to our practical information about the yeasts, and led to the now universal practice of using in fermentation industries pure strains of yeasts, cultivated in the laboratory and especially selected for their efficiency in bringing about a particular type of fermentation.

The most important of the fermentative changes produced by yeasts is the breaking down of sugar to form *alcohol* (so-called alcoholic fermentation). The reaction is represented by the following equation:

$$C_6H_{12}O_6 \text{ (sugar)} \rightarrow 2C_2H_5OH \text{ (alcohol)} + 2CO_2 \text{ (carbon dioxide).}$$

Formation of alcohol goes on in this way when living yeasts grow in a sweet solution, for example in the juice of ripe grapes. Either the wild yeasts naturally present on the grape skins, or cultivated yeasts deliberately added to the mixture, may be used to bring about the alcoholic fermentation, but the latter are preferable because especially chosen wine yeasts are not only good alcohol formers, but they are resistant to rather high concentrations of alcohol, so that they continue to be active while the alcohol content climbs to 10 or 12 per cent or higher. The actual transformation of sugar to alcohol occurs through the action of the enzyme "zymase," which may be expressed from the yeast cells by pressure.

Saccharomyces cerevisiae is the name most often applied to the common bread, beer and wine yeasts.

The familiar yeast cake is composed of compressed living yeast cells, mixed with a little starch. In the making of bread, the first gentle heating of the dough causes the added yeasts to multiply. They ferment the sugar present and cause the release of carbon dioxide. This harmless gas escapes through the dough, leaving countless small holes. Thus the dough rises and the bread becomes light and porous.

Fresh cells of these harmless yeasts are rich in Vitamin B (B_1) and also contain Vitamin G (B_2).

Torulae. These are distinguished from the yeasts above described by the fact that they fail to form ascospores (or other types of spores) under any circumstances. They also differ in other respects. They are generally smaller, spherical cells, less widely distributed, and not of practical use in fermentation industries. The most commonly encountered of the harmless saprophytic torulae are the so-called red torulae. These are seen not infrequently as contaminants in bacterial cultures and are especially numerous in cream and other dairy products. Their colonies on solid media are sticky, mucoid, and have a red or pink color.

The medical importance of these organisms arises from the fact that a species of torula, called *Torula histolytica* is highly pathogenic for human beings, and may cause a fatal infection of the brain (Chapter XL).

FUNGI PATHOGENIC FOR MAN

The pathogenic fungi are not easy to classify. It is possible, however, to arrive at a satisfactory working classification if we base the arrangement in part on the properties of the organisms, and in part on the clinical features of the diseases they produce. Since the fungous diseases are considered more fully in Chapter XL, we give here only a brief outline of the chief disease-producing fungi. There are twelve varieties or groups of these organisms as follows:

(1) **Aspergillus fumigatus.** A typical mold of the genus *Aspergillus*, causing *aspergillosis*, a comparatively rare infection of the external ear or lungs.

(2) **Sporotrichum schencki.** A fungus appearing as small oval or cigar-shaped bodies in pus, and growing in culture as a branched, septate mycelium with pear-shaped spores arising directly from the sides and tips of the hyphae. The cause of *sporotrichosis* in man.

(3) **Microsporon furfur.** Cause of *pityriasis versicolor* (tinea versicolor), a common disease of the superficial layers of the skin, characterized by a brownish discoloration. This fungus appears in the lesions as a closely interlaced mass of irregular filaments with many spores.

(4) **Microsporon minutissimum** (*Actinomyces minutissimus*). An organism growing as narrow, branched threads. It causes *erythrasma*, an affection of the moist skin, characterized by a reddish brown discoloration.

(5) **Ringworm fungi (Dermatophytes).** Several species of the genus *Trichophyton* and *Microsporum*, causing *ringworm* of the skin or scalp. The fungi appear as filaments and chains of spores within or around the hair shafts.

(6) **Achorion schoenleini.** Infects the scalp, smooth skin, or nails, producing clinical condition (rare in U. S.) called *favus*. Lesions are characterized by yellowish cup-shaped masses (consisting of tangled filaments) about the base of the infected hairs.

(7) **Epidermophyton cruris.** Cause of an affection of the moist areas of the skin known as *eczema marginatum* or tinea cruris. In the tropics this infection is called *dhobie itch.* Another variety, *E. interdigitalis,* is one of the several fungi that may be responsible for the common condition known as "athlete's foot."

(8) **Madurella mycetomi.** One of the common causes of "madura foot," a tropical disease occasionally seen in America. In the lesions the organisms appear in the form of black granules composed of masses of a large, septate mycelium.

(9) **Coccidioides immitis.** This fungus appears in tissues as a large, thick-walled, cyst-like body, without buds, but grows in cultures with an abundant mycelium. Cause of *coccidioidal granuloma,* a severe and usually fatal generalized infection.

(10) **Blastomyces hominis** (also called *Blastomyces dermatitidis, Oidium dermatitidis,* and several other names). This fungus appears as a yeast-like budding form in the tissues, but forms mycelium in cultures. Cause of *blastomycosis,* a disease which takes several clinical forms, involving the skin primarily in some cases and the lungs in other cases, and often becoming generalized.

(11) **Monilia albicans** (also called *Oidium albicans*). This name is most commonly applied to the several varieties and sub-varieties of yeast-like fungi that may infect the mucous membranes in man. They grow both in the tissues and in laboratory cultures as single, budding cells, but in addition may at times form a rudimentary mycelium. They cause *thrush,* an infection of the mouth in young children, are abundant in the feces in cases of tropical sprue, and are also encountered in a variety of other disease conditions, spoken of collectively as the *Moniliases.*

(12) **Torula histolytica** (also called *Cryptococcus hominis,* or *C. histolyticus*). A yeast which multiplies by budding, forms no mycelium and no spores. Cause of a fatal brain infection and other forms of *torulosis.*

THE MOLD-LIKE HIGHER BACTERIA

This is a large and important order of microbes known as the *Actinomycetales,* in which are grouped a variety of microorganisms having properties resembling both the true fungi on the one hand and the true bacteria on the other. Some well known members of the group might be called with equal propriety bacteria-like fungi or fungus-like bacteria. Authorities are generally agreed, however, that these organisms are best classified as a subdivision of the bacteria (*Schizomycetes*), hence they are customarily spoken of as the *mold-like higher bacteria.* As we indicate in the diagram on page 46, there are two principal families: (1) *Actinomycetaceae* and (2) *Mycobacteriaceae.*

Actinomyces. This is the most important genus of the *Actinomycetaceae,* and contains the most mold-like of the higher bacteria. The *Actinomyces* grow in the form of branched filaments, thus forming a true mycelium like that of a common mold, and moreover, sometimes short aerial hyphae extend into the air above the growth and from the tips of these are pinched off round or rod-shaped reproductive elements (conidia) like the arthrospores of some true fungi. When the aerial mycelium and the spores are well developed, the surface of a colony on a solid medium has that powdery look which we associate with typical molds. The saprophytic species found in the soil and elsewhere are especially characterized by this mold-like development.

But even the most mold-like forms differ in several respects from the typical molds, and many varieties, especially the pathogenic strains, have marked similarities to the true bacteria. The mycelium is always made up of fine, *narrow* filaments, only about 1 μ wide, (i.e., about the width of most bacterial cells), and therefore much thinner than the hyphae of molds. Also the filaments are homogeneous throughout, without cross walls, or any visible internal structures. Moreover, most *Actinomyces* show early in their growth a *fragmentation of the mycelium* into round or rod-shaped segments that have the size and aspect of ordinary bacteria. Smears from actively growing cultures of this kind reveal only *bacteria-like elements* of various shapes. scattered through the field (Figs. 11:8; 17: C).

The word *actinomyces* means "ray-fungi," and was suggested to Harz, who originally named these organisms in 1877, by the appearance of the organisms in the sulphur-yellow *granules* that appear in the discharge from tissues infected with the pathogenic variety *Actinomyces bovis.* When a granule is crushed under a cover glass and examined it appears as a rosette-like mass, made up of a dense network of filaments which radiate outward from the center. The free ends of the filaments at the outer edge of the granules are thickened and lobulated, forming what are called "clubs." The central mass of filaments takes the basic stains, but the peripheral clubs stain with acid dyes. The thickened, clubbed ends are probably brought about by the reaction of the living organism to the body fluids which surround the granules, the tip of each filament becoming encased in a clear, acid-staining material. These granules develop in the tissues only, and are not seen in laboratory cultures (Fig. 96).

Unlike the true fungi, the actinomyces are sensitive to acid, and require a neutral or alkaline culture medium, such as would be used for the ordinary bacteria. The pathogenic species are cultivated in the laboratory with difficulty, and for them blood agar and other enriched media like those used for pathogenic bacteria are necessary.

Saprophytic actinomyces. As much as 30 per cent or more of the micro-organisms that may be cultivated from the soil consist of various species of *Actinomyces.* These organisms probably play an important rôle in the decomposition of dead plant and animal matter. Since these saprophytic varieties may produce spores (conidia) which get into the air (Fig. 17) it is not surprising that their colonies sometimes appear as contaminants on Petri dish cultures. These colonies are usually recognizable by their tough, almost cartilaginous consistency, their close adherence to the agar, and the brownish discoloration in the surrounding medium.

Pathogenic actinomyces. There are several varieties of *Actinomyces* that are pathogenic for man and animals. The infections caused by these microbes may take many clinical forms, and may involve various regions of the body. For practical purposes, however, we may recognize three pathogenic species and three main types of disease, as follows:

(1) **Actinomyces bovis.** The most important species, cause of *actinomycosis* in cattle and in man, an infection involving characteristically the tissues of the neck at the angle of the jaw (called "lumpy-jaw" in cattle), or, less commonly, the lungs or intestine. This organism grows best in anaerobic, or partially anaerobic conditions (see Chapter XL).

(2) **Actinomyces madurae.** Cause of some cases of *madura foot* and other forms of *mycetoma,* seen mostly in the tropics (other cases are due to the mold *Madurella mycetomi,* as noted above).

(3) **Actinomyces asteroides.** This species and related aerobic strains cause relatively rare infections of the lungs, wounds, or other tissues, which may resemble tuberculosis clinically. These organisms are *acid-fast,* like the tubercle bacilli.

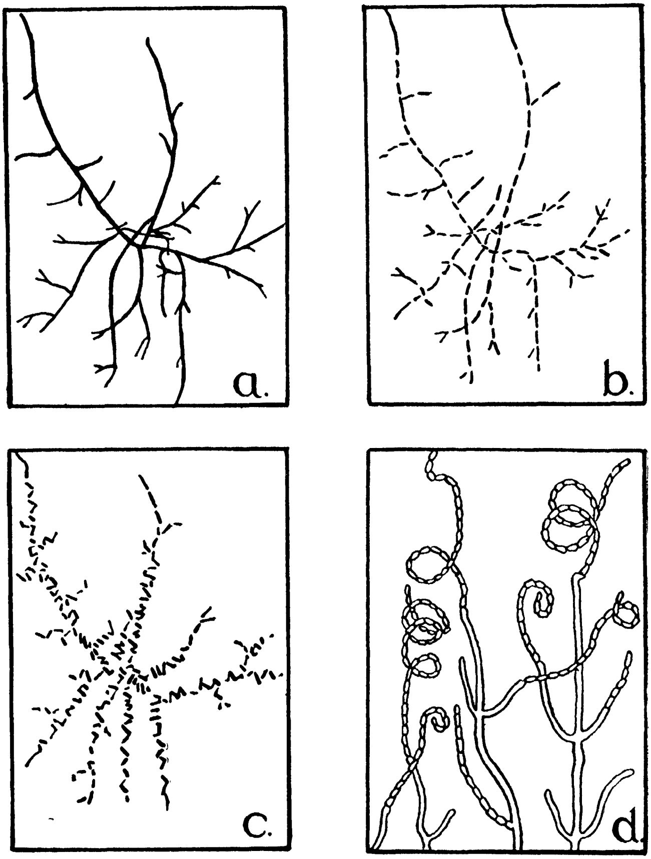


FIG. 17. Actinomyces. A, b, c, stages in the development of a pathogenic species, showing fragmentation of the mycelium (b) and the subsequent shift in the position of the bacteria-like fragments (c) so that they lie more or less parallel, resembling diphtheroidal bacilli; d, the twisted, terminal, conidia-bearing filaments of a saprophytic actinomyces. (Redrawn after Henrici.)

Mycobacteriaceae. In this family are classified those "higher bacteria" that seem to occupy a position intermediate between the *Actinomyces* and the simpler "lower bacteria." They are less mold-like than the *Actinomyces,* but more complex in cell structure and development than the great bulk of "ordinary," "lower" or "true" bacteria. The limits of the group cannot be sharply defined, for there is no one characteristic which determines the classification of any bacterium into the higher or the lower order. All authorities agree, however, to place here certain of the most important disease germs. The four genera usually recognized within this group, and the principal species in each genus are listed in the following paragraphs.

Mycobacterium (acid-fast bacilli). The organisms of this genus are slender, Gram-positive, nonmotile rods. When once stained fully by carbol fuchsin they resist the decolorizing action of acids, hence are said to take an *acid-fast* stain. The cells are often irregular in length and outline, showing swollen ends or a beaded appearance.

Mycobacterium tuberculosis (hominis) is the bacillus of human tuberculosis.

Mycobacterium tuberculosis (bovis) or *Myco. bovis* is the bovine (cattle) tubercle bacillus.

Mycobacterium avium is the bacillus of avian (bird) tuberculosis.

Mycobacterium piscium is the cause of a tuberculous disease in fish and other cold-blooded animals.

Mycobacterium leprae is the germ of human leprosy.

Mycobacterium smegmatis is a harmless species found in the smegma of man and animals.

Mycobacterium phlei, the timothy grass bacillus, and *Mycobacterium butryicum,* the butter bacillus, are examples of a large group of harmless, saprophytic acid-fast bacilli found everywhere in nature.

Corynebacterium. These organisms are slender, Gram-positive, nonmotile, sometimes slightly curved rods, *not* acid-fast. They occur typically in groups in which the cells have a more or less perfect parallel arrangement. The individual bacilli vary greatly in size and shape, and show characteristic irregularities of staining with conspicuous metachromatic granules.

Corynebacterium diphtheriae is the diphtheria bacillus, the specific cause of diphtheria.

Corynebacterium hoffmanni and *Corynebacterium xerosis* are examples of a large group of so-called *diphtheroids*, which are common as harmless parasites on the human and animal body and as saprophytes widely distributed in nature.

Fusiformis. This genus contains *anaerobic*, Gram-negative, spindle-shaped bacteria, often showing granules and filamentous forms. They are parasitic on the mucous membranes of the mouth and genitalia in man and animals, and are found in infections of the mouth and elsewhere in association with Vincent's spirochete (*Treponema vincenti*). There are probably several species, not at present well differentiated. Usually these organisms are referred to under the name *Fusiformis dentium*.

Loefflerella. This is the same genus that has been commonly called *Pfeifferella*. Only two closely related species, both pathogenic, are included here:

Loefflerella mallei, the bacillus causing glanders in horses and in man, and

Loefflerella whitmori, the cause of melioidosis, a glanders-like disease in man.

The organisms are Gram-negative, aerobic bacilli, which show many irregularities in shape, arrangement and staining.

REVIEW QUESTIONS—CHAPTER VI

1. What are the *Eumycetes?* What common types of microbes are included, and what is their importance?
2. Describe the general structure of molds and of yeasts. Explain the proper use of the terms *mold*, *yeast*.
3. Name the four classes of fungi. What are the principal points upon which this classification is based?
4. Characterize briefly the *Phycomycetes* and the *Basidiomycetes*. Mention some kinds of fungi belonging to each class.
5. Characterize briefly the *Ascomycetes*, and mention common types of fungi belonging to this class.
6. What are the *Fungi imperfecti* or *Hyphomycetes?* Why is their study and classification difficult?
7. Define the following terms and find examples of each in actual specimens or in drawings: *hypha*, *mycelium*, *septate mycelium*, *nonseptate mycelium*, *submersed hyphae*, *aerial hyphae*, *fertile hypha*, *fruiting body*, *sporophore*, *sporangium*, *columella*, *sterigmata*, *phialides*, *blastospore*, *chlamydospore*, *arthrospores* or *oidia*, *sporangiospores*, *conidia*, *zygospores* and *ascospores*.
8. What is a *sclerotium?* Describe a structure of this kind which is the source of the drug ergot.
9. To what four genera and to what classes of the fungi do the majority of the common molds belong?
10. Name and describe a common species of the genus *Rhizopus*.

11. Name and differentiate from *Rhizopus* molds, a common species of the genus *Mucor*. What type of sexual spores are formed by these molds?
12. Name and describe a common species of *Aspergillus*. Why are the aspergilli of special medical interest?
13. Name and describe a common species of *Penicillium*. Name two other species of interest.
14. Discuss the practical importance of the common molds, illustrating by examples their undesirable and harmful effects, and also their helpful activities. Why are the common molds of medical interest?
15. Where are the yeasts found? How do the true yeasts differ from other fungi? What is the probable relationship of the yeasts to the filamentous fungi?
16. Describe the usual shape and structure of yeast cells.
17. Describe the method of reproduction of yeasts.
18. How do yeasts differ from bacteria?
19. What are the two principal groups among the yeasts?
20. Why are the *Saccharomyces* important commercially? Explain what is meant by "alcoholic fermentation." Name the most common variety of fermenting yeast.
21. Of what is the familiar yeast cake composed? How do yeasts act in making bread?
22. What distinguishes the *Torulae* from other yeasts? Mention some saprophytic varieties.
23. What is the medical importance of the *Torulae?*
24. In the text the pathogenic fungi are listed in twelve numbered groups, and fourteen fungous diseases are mentioned. How many can you recall?
25. What are the *Actinomycetales* and how are they related to other microbes? Name the two principal families.
26. Describe the *Actinomyces*, and compare them with the true fungi and with the ordinary bacteria.
27. What is the literal meaning of *actinomyces* and why is it an appropriate name when applied to the pathogenic *Actinomyces bovis?*
28. What is the importance of the saprophytic *Actinomyces?*
29. List the three principal varieties of pathogenic *Actinomyces* and the disease conditions caused by each.
30. Characterize the family *Mycobacteriaceae*. Name four principal genera included in this group.
31. What five important disease germs are classified here as "higher bacteria"?

CHAPTER VII

THE SPIROCHETES, RICKETTSIAE, AND FILTRABLE VIRUSES

THE SPIROCHETES (SPIROCHETALES)

THE SPIROCHETES make up an order of microbes of great interest and importance. They have some properties that suggest protozoa, but most authorities classify them as a higher order of bacteria. The word *spirochete*, which means "coil of hair," is appropriate, because all of these organisms are shaped like coiled hairs or threads.

There are many kinds of spirochetes, and as in other classes of microbes, most species are harmless, but a few kinds cause serious and common diseases.

Principal groups. For practical purposes, four groups among the spirochetes may be recognized (Fig. 18). These are:

(1) Free-living saprophytic forms, entirely harmless, found in stagnant water, cesspools, and damp soil. These are placed in the genus *Spirocheta.*

(2) Relatively large, worm-like forms found within oysters and other shellfish (genus *Cristispira*), and related free-living varieties (genus *Saprospira*). These are harmless for human beings.

(3) Minute spirochetes of various sizes and shapes *found normally in the healthy mouth, on the genitals, and in the intestinal tract of human beings and animals.* These are harmless parasites under ordinary conditions, though some species are found frequently, associated with other microbes, in inflammatory processes in various parts of the body. Most of these spirochetes are placed in the genus *Treponema.*

(4) *Pathogenic spirochetes* which invade the blood (as in relapsing fever), the tissues (as in syphilis), or both the blood and the tissues (as in infectious jaundice). These belong respectively to the genus *Borrelia*, *Treponema*, and *Leptospira.*

General characteristics of the parasitic spirochetes. As can be seen in Fig. 18, the parasitic spirochetes are relatively small and delicate, as compared with the saprophytic forms. They have no head, nor tail, and they are so thin that it is not possible to discern any internal parts. The different varieties of these curious microbes are principally distinguished from each other by the number and character of their spirals, and by differences in the speed and manner of their movements. Their true shape is probably considerably distorted in stained smears, and they are best studied in the living state, in materials freshly obtained from the body, under a *dark-field microscope.* Here they appear as delicate, silvery, coiled threads, and each variety shows a more or less characteristic type of locomotion.

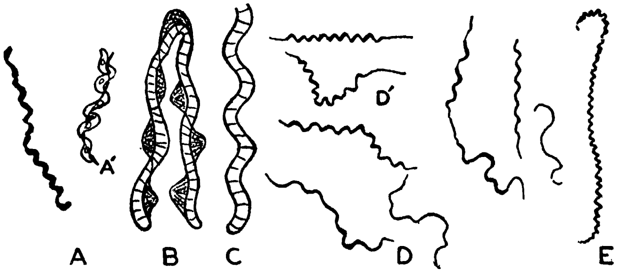


FIG. 18. Spirochetes. A, *Spirocheta.* A′, detail showing the curious spiral structure of these organisms. B, *Cristispira,* showing the spiral crest running along the body. C, portion of a *Saprospira.* D, forms of *Treponema.* D′, the syphilis spirochete, *Treponema pallidum.* E, *Leptospira.*

The spirochetes as a group do not color readily with the ordinary stains used for the true bacteria. The stronger aniline dyes, such as gentian violet and carbol fuchsin, will stain the spirochetes normally found in the mouth and on the genitalia fairly well, but other spirochetes, notably the syphilis germ, *Treponema pallidum,* remain entirely uncolored by these simple stains. For these species special methods are employed (Appendix B).

The *Leptospira* grow readily in various ordinary laboratory media. The relapsing fever spirochetes (*Borrelia*) also may be cultivated readily from the blood of human patients or experimentally inoculated animals. The *Treponema,* however, are so fastidious in their requirements, and so fragile when removed from their natural habitat in the living host that their cultivation in the laboratory is most difficult. They are *anaerobic*, and will grow only in a medium from which free oxygen is excluded. Several of the parasitic *Treponema*, generally recognized to be distinct varieties, have never been grown in pure culture.

All the spirochetes reproduce by a simple division of the spiral across the short axis into two threads of approximately equal length.

The parasitic spirochetes as a group are more readily destroyed than the true bacteria. They are killed quickly by drying, or by exposure to cold, moderate heat, and dilute disinfectants. A striking characteristic is their susceptibility to arsenical compounds (like *salvarsan*), and bismuth, when within the human body. This has made possible the successful treatment of syphilis with these chemicals.

Spirochetes of the mouth and genitalia. On the gum margins in the normal mouth, in tonsillar crypts, on the external genitalia, and in the intestinal tract there are always to be found a considerable number of spirochetes. These are ordinarily harmless commensals, but they are of genuine medical interest because apparently the identical organisms are commonly encountered in large numbers, mixed with others, in certain infections of the mouth, throat, lungs, head, or genitalia.

The following are the more generally recognized forms found in the mouth.

Treponema microdentium. This is a small, thin spirochete, found regularly in the normal mouth. It is about 3—8 μ long, and 0.2—0.25 μ wide. The body tapers to finely pointed ends. Young organisms have about 6, and older forms 12 or more, shallow, slightly angular, regular, spirals. In shape it is similar to *Treponema pallidum.* It shows marked rotary motion and makes more or less rapid progress across the microscopic field by sinuous movements, in which the primary spirals remain rigid. It is not pathogenic.

Treponema macrodentium. This is a medium-sized, relatively thick form, found in the normal mouth. It averages about 12 μ in length, with 6 or 8 spirals, and is 0.4 to 0.8 μ thick. It resembles *Treponema microdentium* in having regular spirals, but these are larger and coarser, and not so rigid. It is probably a harmless commensal.

Treponema buccale. A poorly defined "species" comprising relatively large, coarse forms with very irregular spirals, which occur in varying numbers in the normal mouth. It varies in width from 0.6 to 0.8 μ and in length from 3 to 25 μ. The ends are pointed and the body appears doubly refractile in the darkfield. Motility may be sluggish or active. It stains well with methylene blue. It is thought by some to be identical with *Treponema vincenti* or with *Treponema refringens* (see below). It has probably never been isolated in pure culture.

Treponema vincenti. This spirochete is somewhat larger and coarser than *Treponema microdentium* and *macrodentium*, and more flexible. It is distinguished by a rapid, serpentine type of motility. When at rest the spirals are fairly regular, but as ordinarily observed, especially in stained smears, the organism appears very irregular in outline, with 3 to 6 flat curves of varying shape. During motion these coils continually form, disappear, and reform. The width is about 0.4 μ and the length from 5 to 20 μ. The ends taper off to sharp points. It stains faintly with methylene blue, somewhat better with the stronger simple stains, such as gentian violet. This organism is found in the ulcerative lesions of *Vincent's angina* and other "fusospirochetal diseases" in which it is associated with fusiform bacilli (Fig. 68).

Spirochetes similar to the above have been found on the normal genitalia. The following variety deserves special mention.

Treponema refringens. This is a rather large spirochete found on the normal human genitalia, and in mixed infections of these parts. It is similar in morphology and motility to *Treponema vincenti* and also resembles *Treponema buccale.* It is readily distinguished from the *Treponema pallidum*, when encountered with this organism in syphilitic lesions, by its greater thickness and by the fact that its spirals are constantly changing. It is usually 15 μ or more long, and about 0.6 μ thick. The middle part of the organism is loosely wavy, but the two ends are more regularly and deeply coiled. It is not pathogenic.

Important pathogenic spirochetes. The spirochetes responsible for specific diseases may be placed conveniently into three categories:

(1) the *Leptospira*, invading both the tissues and the blood stream and causing diseases in which jaundice is a feature, transmitted through contaminated water and soil, (2) the *Borrelia*,

infecting the blood and producing fevers of the relapsing type in man and animals, transmitted by blood-sucking insects, and (3) the *Treponema*, causing chronic lesions in the tissues, as in syphilis and yaws, and transmitted by direct personal contact.

Leptospira icterohemorrhagiae is the species responsible for *infectious jaundice* (Weil's disease). This is an epidemic disease clinically resembling yellow fever, characterized by abrupt onset, with fever and other signs, and the eventual appearance of jaundice. In the early stages, the leptospira are present in the circulating blood, but later they disappear from the blood, while they remain in the enlarged liver and other internal organs, and are excreted in the urine.

The organism was first described by Inado, Ido, and their collaborators in 1916. It is 5–12 μ long, and about 0.15 μ thick. In motion, this spirochete shows coarse undulations of the body, but the fine, closely wound, primary coils remain intact. The ends are characteristically hooked, giving the whole organism the shape of the letter S or C. Active rotary motion makes the hooked ends appear like slits or buttonholes in the rapidly rotating thread.

Infected *rats* form the natural reservoir of the *Leptospira*, and the water in coal-mines, trenches, sewers, and the like, becomes contaminated with the spirochetes by the urine of these animals. Infection in man probably occurs through the rubbing of contaminated moist soil into the skin, nose, or eyes, or as a consequence of swallowing polluted water.

Weil's disease is most common in Japan and South America. Many cases occurred among soldiers in the trenches during the World War. Occasionally the infection has been reported in the United States.

Borrelia recurrentis and **Borrelia duttoni** are the principal species in a group of related spirochetes that cause *relapsing fever*. This is a severe disease characterized by a period of fever lasting a few days which ends abruptly, and is followed by a week or more without fever, then a relapse with return of the fever. During the febrile periods the spirochetes are numerous in the blood stream.

Borrelia recurrentis is cause of the European type of relapsing fever, which is transmitted from one person to another by lice. This organism was the first pathogenic microbe to be demonstrated in the blood of human patients when Obermeier described it in 1868. The individual spirochetes are of variable size, averaging about 15 μ in length, and 0.4 μ in width.

Borrelia duttoni is the cause of the relapsing fever prevalent in Africa. It is usually carried from man to man by ticks.

Spirochetes isolated from cases of relapsing fever in North America have been called *Borrelia novyi,* but probably they do not represent a separate species. In the past, epidemics of relapsing fever occurred in the United States, but at the present time, this infection is very rare here. It is now confined almost entirely to warmer countries, and is most common in Eastern Europe, India, and Africa.

Treponema pallidum is the specific cause of the most important of all the spirochetal diseases, namely *syphilis.* A similar spirochete, **Treponema pertenue,** is responsible for *yaws,* a tropical disease resembling syphilis. Discussion of syphilis is reserved for Chapter XXXIV.

THE RICKETTSIAE

The organisms now known as *Rickettsiae* were first observed in 1909 by Ricketts, who saw them in the blood of patients with Rocky Mountain spotted fever. Although the germs looked like small bacteria, they would not grow out on artificial media. Similar bodies were again found by Ricketts and Wilder, in 1910, in smears from the blood of patients with typhus fever, and from lice that had fed on typhus patients. These findings were confirmed by Prowazek during his study of typhus in Siberia in 1913, and shortly thereafter by other investigators in other parts of the world. In 1916 De Rocha-Lima introduced the name *Rickettsia,* when he designated the causative organism of typhus fever *Rickettsia prowazeki* in honor of Ricketts and of Prowazek, both of whom had died of typhus while investigating the disease. The rôle of rickettsiae in causing both Rocky Mountain spotted fever and typhus was further confirmed by the thorough studies of Wolbach and his associates in 1922.

Distinguishing characteristics of the rickettsiae. The rickettsiae are definitely living organisms, but their proper place in relation to other microbes is uncertain. In appearance, they suggest very small bacteria, and it is generally believed that they may have been derived originally from ordinary bacteria. As now found, however, they have a number of peculiarities of their own, which entitle them to recognition as a special class of microorganisms.

The outstanding properties which distinguish the pathogenic *Rickettsiae* as a group are: (1) their minute size (the longest are under 2 μ), characteristic staining, and great variability of shape (Figs. 11:11; 19), (2) their peculiar habitat in certain insect carriers, (3) their apparent development in the infected human or animal body only within living tissue cells, and (4) their complete failure to grow in the laboratory in lifeless artificial culture media. The diseases caused by these organisms all have similar features, and differ in several respects from most infections due to ordinary bacteria.

There are good grounds for the assumption that the rickettsiae were originally insect parasites only, and have become secondarily parasitic in various mammals and in man through the agency of blood-sucking insect vectors. In all of the rickettsia diseases the causative organisms, at some time in the cycle of transmission from host to host, pass through the bodies of insects.

The classical epidemic European typhus is carried from man to man by infected body lice. The lice themselves are eventually killed by the rickettsiae. Endemic typhus, or murine typhus, a milder form occurring in the United States and elsewhere, for which rats are the natural reservoir, is transmitted from rat to rat, and from rat to man by infected rat fleas. Rocky Mountain spotted fever is spread by the bite of ticks. The rickettsiae of the latter disease may be carried from one generation of ticks to another through the eggs; the organisms are thus kept alive in these creatures without necessarily passing through a mammalian host.

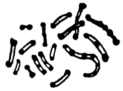


FIG. 19. Rickettsiae. The curious bacteria-like bodies found in the gut of lice, ticks, and other blood-sucking insects, and responsible for typhus fever, trench fever, and Rocky Mountain spotted fever. (Adapted from Wolbach, Todd, and Palfrey, *Etiology and Pathology of Typhus.* Courtesy, Harvard University Press.)

As we now encounter them the pathogenic rickettsiae are all highly specialized *intracellular parasites.* They may be found in the blood and in all of the organs of the infected individual, but they are much more abundant within the cytoplasm (or nucleus) of certain cells, such as those lining the peritoneum or the walls of the small blood vessels. They apparently multiply only within suitable living body cells.

Cultivation. We cannot grow the pathogenic rickettsiae in lifeless laboratory media. They may be cultivated, however, in artificial tissue-cultures, where they develop within the growing cells of the culture, just as they do under natural conditions in the body. They also may be grown on the chorio-allantoic membrane of the embryo chicken by the procedure successfully used for the propagation of the filtrable viruses (described below), and in the yolk sac of the growing chick.

When rickettsiae are inoculated into the peritoneal cavity of male guinea pigs or rats, the organisms accumulate in the cells of the delicate tunica vaginalis (the fold of the peritoneum that envelops the testes), and large numbers of them may be collected from this tissue for study, or for the preparation of vaccines. An especially abundant development is secured in rats whose resistance has been lowered by previous exposure to x-rays.

Species of pathogenic rickettsiae. A variety of rickettsia was recognized (1916) as the probable cause of *trench fever*—a disease occurring among troops during the World War, and another species was named (1923) as the cause of *tsutsugamushi disease*, a typhus-like illness prevalent in certain regions of Japan. The only varieties of importance in the United States, however, are those causing *typhus* and *Rocky Mountain spotted fever.*

Rickettsia prowazeki is the species responsible for both of the two principal types of human *typhus:* (1) the classical European disease whose natural reservoir is man, and which is transmitted by lice, and (2) the endemic or murine type occurring in Mexico, the southern United States, and elsewhere, whose natural reservoir is in rats, and which is transmitted mainly by the rat flea.

Rickettsia rickettsi (*Dermacentroxenus rickettsi*) is the cause of *Rocky Mountain spotted fever* of both the western and eastern types as seen in the United States, and elsewhere. It is transmitted principally by the wood tick *Dermacentor andersoni,* and by the dog tick *Dermacentor variabilis.*

Typhus fever and Rocky Mountain spotted fever are discussed n Chapter XXXVIII.

FILTRABLE VIRUSES

Discovery of filtrable infectious agents and of inclusion bodies and elementary bodies. We have already described how the invention of filters which do not allow the passage of ordinary bacteria led to the discovery that the causative agents of certain diseases will pass through such filters in invisible form. The first to observe this was Iwanowski in 1892 when he found that the agent responsible for the mosaic disease of tobacco leaves is filtrable. This was proven in the following manner. Some of the diseased leaves were emulsified in water. Then this emulsion was filtered through a sterile stone filter similar to those used in bacteriological laboratories today. *The ordinary bacteria do not pass through such a filter.* The *filtrate* from this emulsion of diseased leaves was found to be entirely free of ordinary bacteria and no form of living thing could be seen when it was examined with the microscope. Nevertheless *when some of this clear filtrate was placed upon healthy tobacco leaves, these leaves soon sickened* and showed every sign of the typical mosaic disease.

In 1898 Loeffler and Frosch in a similar way demonstrated that an infection of animals sometimes seen in man, called foot and mouth disease, is also caused by a filtrable, invisible "virus."

Since these pioneer studies a large number of other disease conditions, including some of the most common and serious of human ills, have been shown to be due to infection with similar, filter-passing agents. In each of these diseases blood samples from the patient or washings from the nasal passages, or extracts from body discharges or from infected tissues, when passed through a bacterial filter yield a filtrate which is free of any organisms visible by ordinary methods of microscopical examination, and which gives no growth of any kind on artificial culture media, yet contains a mysterious element—the virus—capable of reproducing the same disease in other individuals. Though the virus can neither be seen nor cultivated by usual methods its presence is proven by the fact that the clear filtrate will cause the appearance of characteristic signs and symptoms of disease when inoculated into a normal, susceptible host.

For want of a better name, the elusive infectious agents of this sort are now generally referred to as *filtrable viruses*, or are called, merely, *viruses*—terms which do not imply any definite notion as to their real nature.

Filtrable virus diseases. At the present time a large number of diseases are attributed to infection with a virus. The number continually increases as new virus diseases are recognized. Some of the more important and best known are listed in the following table.

SOME FILTRABLE VIRUS DISEASES

In Bacteria
Transmissible lysis (action of bacteriophages)

In Plants
Mosaic diseases of tobacco, tomato and potato plants
Ring-spot of tobacco leaves
"Breaking" of the solid color of normal tulip petals

In Insects
"Silkworm jaundice" (an affection of the caterpillar of the silkworm moth) and similar diseases of other caterpillars

In Animals

Hog cholera
Distemper in dogs
Foot and mouth disease
Vesicular stomatitis in horses
Sheep pox
Fowl pox
Rabies
Pseudorabies
Fox encephalitis
Encephalomyelitis of horses
Louping ill of sheep
Psittacosis in parrots
Swine influenza
Virus III infection of rabbits
Myxomatosis and fibromatosis of rabbits
Infectious ectromelia in mice
Rous sarcoma of chickens
Fowl leukemia
Fowl plague
Cattle plague
Salivary gland disease of guinea pigs

In Human Beings

Smallpox
Chicken-pox
Herpes simplex
Herpes zoster
Molluscum contagiosum
Common warts
Measles
German measles
Mumps
Influenza
Rabies
Poliomyelitis
Encephalitis lethargica, and other clinical forms of encephalitis, including St. Louis encephalitis
Lymphocytic chorio-meningitis

Psittacosis	Louping ill
Dengue fever	Lymphogranuloma inguinale
Yellow fever	Foot and mouth disease
Common colds	

Diversity among virus diseases; the distinctive character of individual viruses. These lists, though still incomplete, indicate how extensive is the field of study concerned with virus infections. It seems probable that no form of life is exempt from virus maladies.

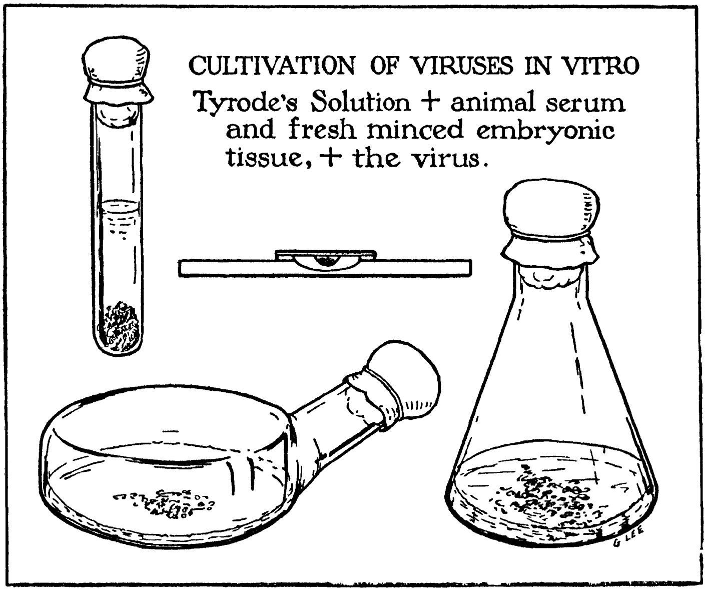

CULTIVATION OF VIRUSES IN VITRO
Tyrode's Solution + animal serum and fresh minced embryonic tissue, + the virus.
G LEE

FIG 20. Methods commonly used for growing filtrable viruses in tissue cultures. The virus multiplies only when the tissue cells are living and of a suitable kind.

Even bacterial cells are subject to injury and lysis (dissolution) by the filtrable agents known as *bacteriophages*. These are so important that we give them special consideration in a later section of this chapter.

The lists serve to emphasize the important fact that there is as much diversity among the virus diseases as there is among bacterial diseases. *The different filtrable viruses are definite entities, differing as sharply from one another as do the separate species of fungi or bacteria.*

The activity of each virus is specific. For example, ordinary measles and German measles, though similar, are nevertheless quite distinct diseases. Virus diseases of insects and animals occur only in certain particular species. Also, in human diseases of this kind, the viruses show a specific action upon certain tissues only. The virus of rabies (hydrophobia), for instance, does no harm until it comes in contact with the tissues of the spinal cord and brain.

Fundamental properties of viruses; a definition. The viruses are universally regarded as a distinct class of infectious agents, and for the specialist in this field the word *virus* has almost as definite a meaning as bacterium or spirochete. The most experienced expert, however, finds it difficult to give a fully satisfactory definition. The evidence is strong that certain of the viruses are *living, ultramicroscopic organisms,* but *others seem to lack the usual attributes of life,* and appear like inanimate substances, in the nature of enzymes. While the question of the living or nonliving character of the viruses is still debated, it is necessary for purposes of definition to fall back upon the few general properties which all the viruses share in common. These may be summarized as follows:

(1) Viruses are smaller than bacteria or rickettsiae and are invisible by ordinary methods of microscopic examination,

(2) They pass through filters which hold back ordinary bacteria (though some of them go through with difficulty),

(3) They cannot be grown in artificial media without the presence of living cells, and

(4) They exist in the most intimate relationship with the living body tissues which they damage, and they multiply (or regenerate) only within, or in close association with, young, susceptible living cells.

The first and last mentioned qualities especially characterize the filtrable viruses as a class. In general terms, then, we may define them as *ultramicroscopic infectious agents, which appear to be intracellular parasites, and are clearly dependent for multiplication upon an intimate association with living cells.*

Isolation, identification, and cultivation of viruses. Despite the natural difficulties in dealing with a material that is entirely invisible by ordinary microscopic examination, and cannot be cultivated in ordinary media, much has been learned about the viruses by laboratory studies.

Isolation of a virus. Animal experimentation is an essential part of laboratory work with viruses, and a necessary step in attempting to isolate a virus is to find a susceptible species of animal in which it may be propagated. The original tissue-extract or other material suspected to contain a virus must first be shown to be free of any visible or cultivable microbes (or it must be made so by filtration through a bacterial filter), then the bacteria-free material must be inoculated into a suitable animal. The resulting appearance of definite illness and pathological changes in the tissues reveals the presence of some kind of virus.

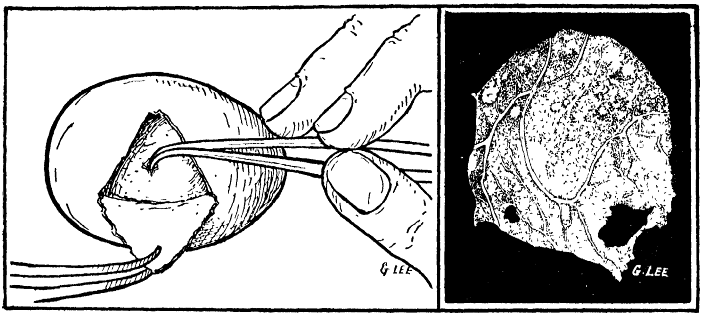


FIG. 21. Cultivation of viruses on the chorio-allantoic membrane of the developing chick embryo. Left, one method of inoculating; a bit of infected membrane from a previous egg culture is being introduced through the exposed shell membrane of the fertile egg on to the chorio-allantoic membrane that lies just beneath. A more usual procedure is to remove entirely a much smaller square of the egg shell, then introduce the fluid virus by capillary pipette or needle and syringe. The opening in the shell is then sealed by a sterile coverslip and paraffin or by paraffin alone. On return of the inoculated egg to the incubator, the virus multiplies on the still-growing fetal membrane of the embryo chick. Right, a chorio-allantoic membrane removed after growth of a virus, showing the white, opaque areas produced in it. From such infected membranes viruses may be recovered readily for further studies.

It is not necessary to show that an infectious agent is filtrable before accepting it as a virus. It is only necessary to prove that it is not visible by ordinary methods of examination, and cannot be cultivated on lifeless artificial media. Although the viruses were originally separated from other types of infectious agents on the basis of their filtrability, the capacity to pass a filter is more of an incidental than an essential attribute of a virus. Of course, in most work with viruses filtration is frequently and regularly used to assure the freedom of the virus material from bacteria, and the significant point is that when properly prepared and suspended in a suitable fluid, viruses *will* pass filters, and appear in active form in bacteriologically sterile filtrates. But filters differ, and so do the conditions of filtration, so that there is no set standard of filtrability.

Identification. The identity of a virus is indicated to some degree by the clinical features of the disease it produces in the experimentally inoculated animal, and to a still greater extent by the character of the gross and microscopic pathological changes it brings about in the infected tissues. But the final proof that one is dealing with a particular virus is furnished by immunological tests. These depend upon the fact that the blood serum from a person who has recently recovered from a virus disease, or from an experimental animal that is immune to a certain virus, will neutralize the disease-producing power of that virus, or will otherwise act upon it and *upon that virus alone.*

So-called *protection tests* are carried out. The blood serum from a person or animal known to be immune to a particular virus and the unknown virus in question are mixed in a test tube and incubated for an hour at 37° C, then this serum-virus mixture is inoculated into susceptible test animals. At the same time control animals are inoculated with the virus alone to prove that it is active. If the test animals remain well, that is, if they are protected by the serum, the virus must be the same as that to which the individual supplying the serum is known to be immune.

Cultivation. Viruses are commonly maintained for study by repeated passage through laboratory animals. Many viruses have been adapted to the brains of mice, and are kept in active state by successively inoculating one lot of mice after another with some of the infected mouse brain material from a previously inoculated lot.

Most of the viruses have also been cultivated in artificial preparations containing living, susceptible tissue-cells. Two methods are in wide use: (1) tissue cultures and (2) egg cultures, utilizing the fetal membrane of the chick embryo (Figs. 20, 21).

Further characteristics of viruses and virus diseases. The application of the methods just described, and other ingenious procedures still more recently developed, have served to remove much of the mystery which formerly surrounded the viruses, and it is possible to outline further positive facts about them, which help us to understand what their real nature may be.

(1) Different viruses, as they naturally occur, have a marked affinity for particular hosts and for particular tissues, but most of them may be induced experimentally to develop in various other hosts and in various other tissues. As we have already mentioned, many viruses, including those of yellow fever and other diseases which do not primarily affect the nervous system, have been made to grow in the brains of mice, and are regularly maintained for study in many laboratories in this way.

In the process of adaptation of a virus to different hosts the original *virulence* (disease-producing power) may be considerably changed. For example, the smallpox (*variola*) virus when inoculated into calves becomes modified into cowpox (*vaccinia*) virus. When now this altered virus is reinoculated into human beings it causes not smallpox, but only the local vaccination skin lesion. Its virulence for man has been greatly reduced. When the virus of rabies obtained from the saliva of a mad dog is first introduced into the brains of rabbits, it is not highly virulent for these animals, but when the virus is repeatedly passed from rabbit to rabbit it becomes so active that a very small dose will invariably cause death in a few days. At the same time the virus from rabbit brain is less virulent for dogs.

Throughout all such manipulations, however, and despite changes in virulence, the *individual viruses maintain their identity.*

(2) The viruses are generally found to be present in very high concentration in diseased tissues, and often the infection may be maintained in susceptible animals with extremely small doses. The abundance of virus in infected individuals perhaps accounts in part for the great communicability of most virus diseases. The viruses are evidently *passed very easily from person to person* and are so *highly infective* that, in times of epidemic, few susceptible persons escape an attack. Practically all children con-

tract measles, for example, as the result of their first contact with other infected children.

(3) The viruses appear to be foreign to the tissues they invade because they are *antigenic*, that is, they lead to the formation of specific *antibodies* (the usual response of the body to foreign matter) and to the development of specific resistance. One of the most outstanding features of the virus diseases as a group is the *high degree of immunity conferred upon those who recover.* It is only very rarely that persons who have recovered from

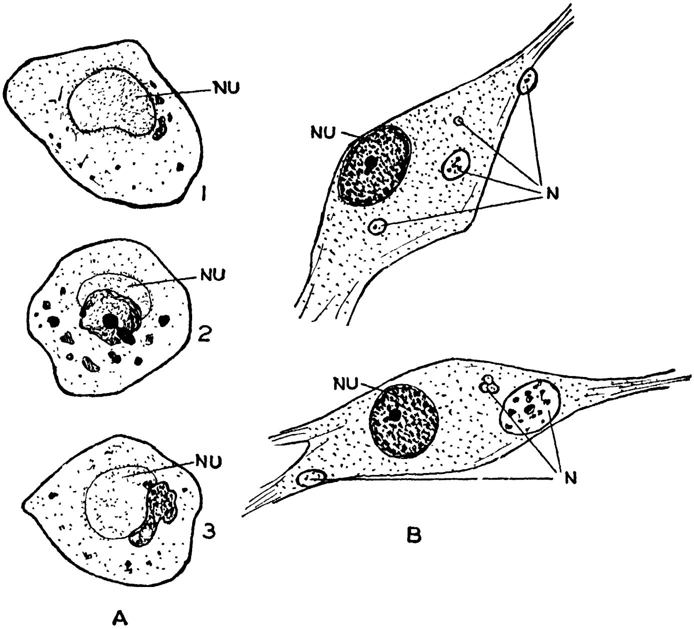


FIG. 22. Examples of "inclusion bodies" which develop in certain body cells in many diseases caused by filtrable viruses. A, *Guarnieri bodies*, characteristic of infection with smallpox virus. 1, 2, 3, cells from the cornea (eye) of a rabbit after inoculation of the eye with smallpox vaccine virus (cowpox virus). The dark, irregular shaped bodies close to the nucleus (NU) are the Guarnieri bodies. These are just beginning to appear in 1, and are further developed in 2 and 3. (After Cowdry, in Rivers.) B, *Negri bodies* in pyramidal cells from the brain of a mad dog. These bodies (N) are a constant and characteristic finding in cases of rabies. (A, adapted from Rivers "Filterable Viruses." Courtesy of Williams & Wilkins Co.)

measles, mumps, smallpox, or other virus infections have the same disease the second time. This is not true in equal degree of many of the infections caused by bacteria and fungi.

(4) In the tissues affected by certain viruses there occurs a remarkable and characteristic change inside some of the cells. When stained, these cells are seen to contain bodies of various sizes and shapes not found in normal cells. These we now call *inclusion bodies.*

As early as 1887 Pfeiffer noticed granular structures in the skin cells affected by smallpox virus. These inclusions were studied more fully (1892) by Guarnieri and have since been known as the *Guarnieri bodies* (Fig. 22). They are constantly found in cells infected with the virus of smallpox or of cowpox. In 1903 Negri described characteristic inclusions in the cells of the brain infected with the virus of rabies—the *Negri bodies* (Fig. 22). Subsequently, inclusion bodies were demonstrated in tissues affected by many other viruses, and we know now that the majority, though not all, of the virus diseases are accompanied constantly by the appearance of some type of inclusion in the particular body cells injured by the virus. *Such inclusions are not found in tissues invaded by ordinary bacteria.* The inclusion bodies in many of the virus diseases have a peculiar form and character, so that when they are seen in any of the body cells, they are a sure sign of the presence there of a particular virus. Diagnosis of rabies in dogs, for example, is regularly accomplished by searching for Negri bodies in the brain cells.

The nature of these inclusion bodies is not quite certain, but modern research supports the theory that they are essentially *colonies of the infecting virus.* Observers noted many years ago that inside the inclusions of smallpox, and of some other virus diseases, numerous very tiny granular elements may be discerned by special methods of microscopic examination, and recent investigations have shown clearly that these granules—the so-called *elementary bodies*—when freed from the inclusion body, act as if they were particles of the virus itself.

By the use of powerful ultracentrifuges, concentrated and purified preparations of the elementary bodies of smallpox virus and of some other viruses have been made (Fig. 23). It has been found that the elementary bodies from any particular virus disease are of nearly uniform size. They are infectious and reproduce the typical disease when inoculated into susceptible animals. They are agglutinated, or are otherwise acted upon specifically, by the serum of individuals known to be immune to that virus.

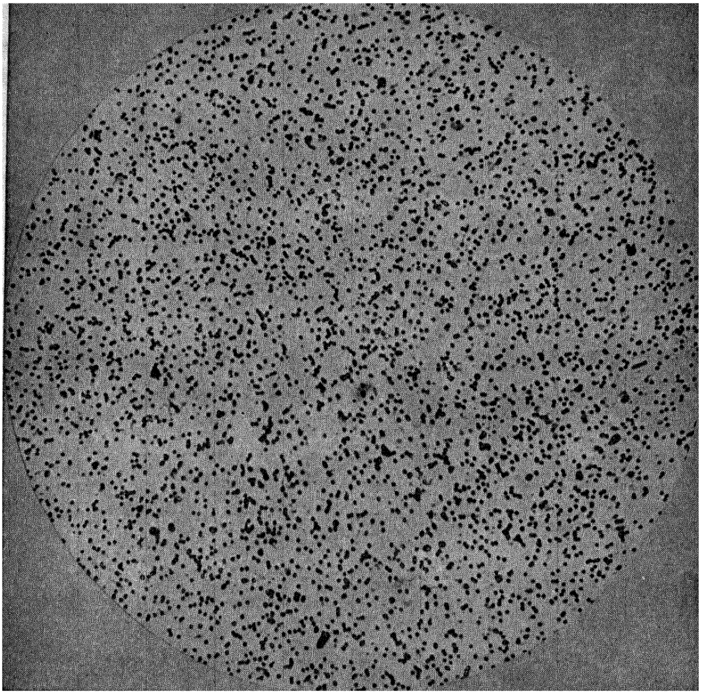


FIG. 23. Elementary bodies of vaccine virus, concentrated by centrifugation, from the experimentally infected chorio-allantoic membranes of embryo chicks. Morosow stain (a silver impregnation method). x 1200. (Photo reproduced through the courtesy of Dr. Joseph E. Smadel, Rockefeller Institute for Medical Research.) (Smadel and Wall, Jr. Exper. Med., v. 66, 1937, p. 325.)

Chemical analysis of concentrated suspensions reveals the presence of the same elements one would find in living microbes. In short, they act as if they were the *active units of the virus itself* and all their known properties are compatible with the view that they are *living midget-microbes.*

Unfortunately we cannot speak so confidently about *all* the

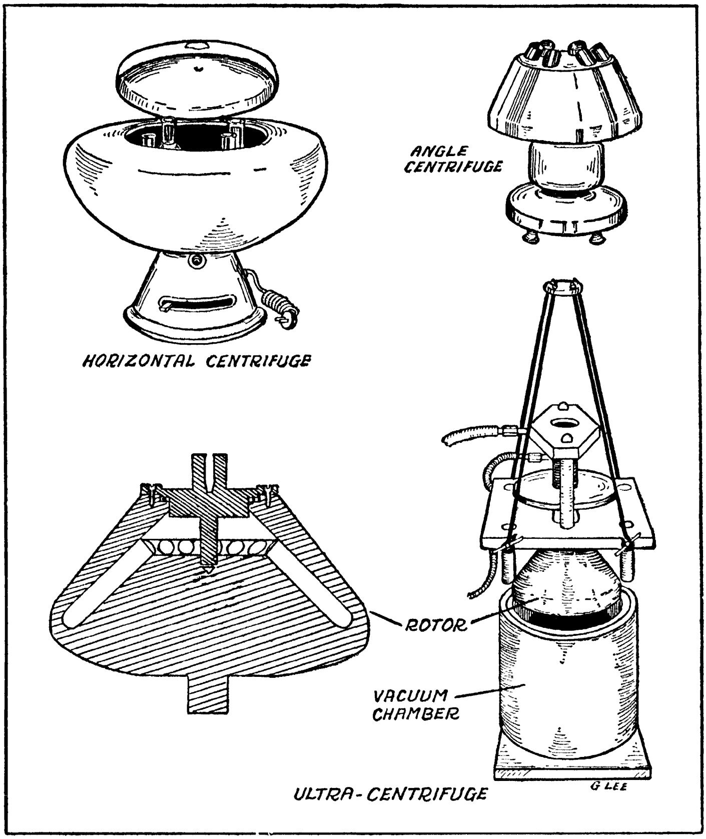


Fig. 24. Centrifuges. In the ordinary Horizontal Centrifuge the tubes are in a horizontal position while spinning. In the Angle Centrifuge they are held constantly at an angle of something less than 50 degrees while they revolve. This makes for greater efficiency, and smaller particles can be thrown down in these newly developed angle centrifuges than could be separated out in the old type centrifuge.

Ultracentrifuges combine the angle principle with great speed; the tubes (made of celluloid because glass would break) are held at an angle within a solid metal rotor, driven by compressed air, which revolves at the rate of 25,000 or more revolutions per minute within a vacuum chamber. Right, the rotor and driving mechanism (simplified) of an ultracentrifuge developed at the Rockefeller Institute, N. Y., shown lifted out of the vacuum chamber. Left, cross section of the rotor. Drawn from the illustrations in the article by Bauer and Pickels, Jr. Exper. Med.: v. 64: 1936: p. 503.

viruses, for no inclusion bodies have been found in such important diseases as poliomyelitis, mumps, and encephalitis, and not all the inclusions that are recognized have been proved to contain infectious elementary bodies.

(5) Whether elementary bodies have been demonstrated or not, there are good reasons for the belief that all viruses are in the form of particles, and moreover, *each kind of virus apparently exists as ultramicroscopic particles having a constant and characteristic size.* Sizes are estimated from the results of ultrafiltration experiments by which the viruses are passed through colloidion membranes having minute pores of known sizes, and from the behavior of the virus materials in the ultracentrifuge (Fig. 24).

In Figure 25 the probable sizes of certain viruses, as determined by ultrafiltration and ultracentrifugation and reported by various investigators, are shown diagrammatically. For comparison the diagram also indicates the sizes of some of the larger protein molecules, and to emphasize the almost infinitesimal dimensions of all these particles they are represented within the outline of a single bacterium—a staphylococcus—itself only 1 micron in diameter. The figures given are expressed in millimicra, or thousandths of a micron (mμ).

The wide range in size of the different viruses, from the 8–12 mμ of the foot and mouth virus to the 300 mμ of the psittacosis virus, is striking. The smallest viruses, which include those of foot and mouth disease, poliomyelitis, and yellow fever, are apparently no more than two or three times the size of a particle of serum globulin. Viruses of medium size include those of encephalitis and of influenza, and also some of the bacteriophages (to be discussed below). The largest viruses are typified by the virus of herpes, lymphogranuloma inguinale, and vaccinia, while the psittacosis bodies are unusually large. It is naturally among the larger viruses that demonstrable elementary bodies have been found. Some of these approach the dimensions of rickettsiae and of certain tiny forms of the bacteria.

(6) Some viruses have been identified with inanimate proteins. Stanley and associates at the Rockefeller Institute (1935), applying the techniques of advanced protein chemistry, succeeded in obtaining the virus of tobacco mosaic disease in what appeared to be chemically pure form. The final product turned out to be

a *protein of high molecular weight.* This substance was in crystalline form, and could be recrystallized repeatedly without losing its activity. Although all possible tests indicated that this material contained nothing but lifeless protein, a very minute amount of it would cause the appearance of the typical mosaic disease when inoculated upon healthy tobacco leaves, and it obviously multiplied, or reproduced itself in some way, in the infected plants. Such a protein could not be isolated from healthy tobacco plants.

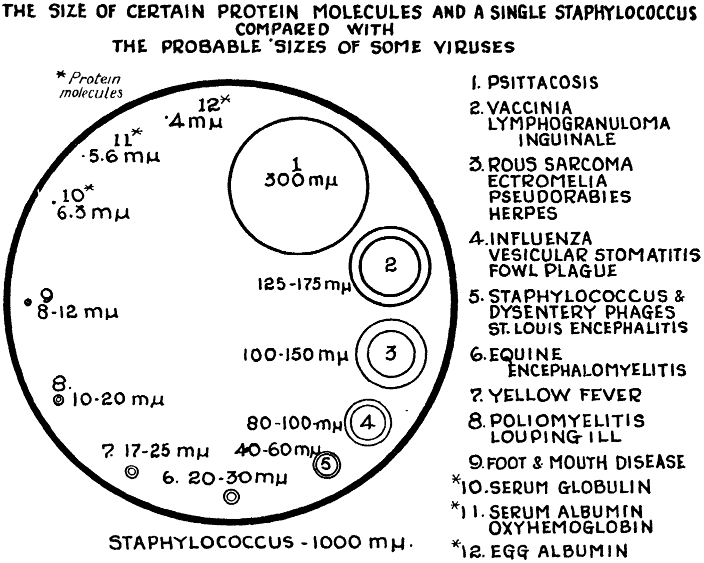


FIG. 25. The large circle represents the outline of a single staphylococcus, having a diameter of one micron, or 1000 mμ. Shown superimposed are smaller circles and dots representing the comparative sizes of some of the viruses and bacteriophages, and certain protein molecules.

More recently other plant viruses, and also certain of the animal viruses, have been separated as heavy proteins. Northop has identified bacteriophages with proteins.

These important findings would seem to indicate that the viruses concerned here (and perhaps others) are not living, but are inanimate, self-reproducing protein substances.

Nature of the viruses. At the present time the true nature of the viruses cannot be definitely decided. While the work of one group of investigators seems to demonstrate that bacteriophages and certain smaller viruses are identical with inanimate proteins, other equally prominent scientists are bringing more and more evidence for the living nature of the vaccinia virus and other large-particle viruses.

It may well be that not all the viruses have the same nature. There is no reason why all the agents that cause infectious diseases should necessarily be living organisms of the types familiar to us.

Rivers has suggested that the larger viruses may indeed be living midget-microbes, of which the elementary bodies are the individual units, while the viruses of intermediate sizes may be forms of life of unfamiliar pattern, on the borderline of organized living cells as we know them, and those of smallest size may be inanimate, self-reproducing incitants of disease.

There is no valid reason, however, for separation of viruses into animate and inanimate on the basis of size alone. In view of all their known properties, most authorities lean to the view that all the viruses, regardless of their size, have something essentially "alive" about them. The character of this "life" in some cases may be different, however, from the organized cells we are accustomed to associate with animate beings.

The viruses seem to have carried dependence upon other living cells to the *n*th degree. Perhaps the ancestors of our present day viruses were larger, living microbes which adopted an exclusively intracellular life, and then, in the course of time, became more and more completely dependent upon their host cells. In consequence, they lost much of their original form and structure so that now some of them consist of no more than a few molecules. At the same time their original physiological activities were largely given up so that now they have little or no independent metabolism, depending upon the vital activities of their living host cells to furnish the few necessary elements they need to maintain and reproduce themselves.

It is a theory only, but an attractive one, which would regard the viruses as a group of "superparasites," which have evolved from larger intracellular parasites but are now reduced to extremely simple form, essentially foreign to the tissues they invade, but existing now only by sufferance of the very cells they damage.

THE BACTERIOPHAGES

Discovery. The English investigator Twort (in 1915) and the French bacteriologist d'Herrelle (in 1917) were the first to call attention to a most remarkable and interesting phenomenon. They discovered that bacteria themselves may suffer a "filterable virus disease." D'Herrelle emulsified a bit of feces from a case of dysentery in broth, then passed the emulsion through a bacteriological filter. He found that the clear filtrate (free of any visible microbes) contained an agent of some kind which would *destroy dysentery germs.* If a trace of this filtrate were added to a young broth culture of dysentery bacilli, all or nearly all of the organisms were dissolved (lysed) and the previously cloudy culture became clear in a few hours. And if a trace of this culture were placed in another young broth culture of dysentery organisms, the same thing occurred, and by successive transfers the mysterious bacteria-dissolving agent could be carried along indefinitely. When a drop of a filtrate containing the unknown agent came in contact with a young growth of dysentery germs on a solid medium, such as an agar slant or plate, the growth became glassy and transparent and the organisms in these transparent areas were found to have been killed. D'Herrelle thought he was dealing with an invisible living microbe which was a parasite upon bacteria. He called this hypothetical ultramicroscopic organism *Bacteriophagum intestinale,* and the term *bacteriophage* (meaning literally "bacteria-eating agent") has come into general use. Often a bacteriophage is referred to simply as a *phage* (Fig. 26).

Chief properties of bacteriophages. Since the original observations of the bacteriophage phenomenon, a great many investigators have studied the subject. It has been found that phages can be isolated from a variety of substances, but are most easily obtained from polluted water or from other material contaminated with the intestinal discharges of higher animals or man. They are always filtrable, that is, they are found in filtrates of various materials, the filtrate always being free of any visible, or cultivable, microbes.

There are many different bacteriophages, and the action of each is specific, that is, it affects a particular type or species of bacterium and closely related types only. Usually strains of bacteria that are alike immunologically (react with the same antibody) are also susceptible to the same bacteriophage. Phages have been isolated for bacteria of many kinds, including saprophytic and nonpathogenic species, as well as parasitic and pathogenic varieties.

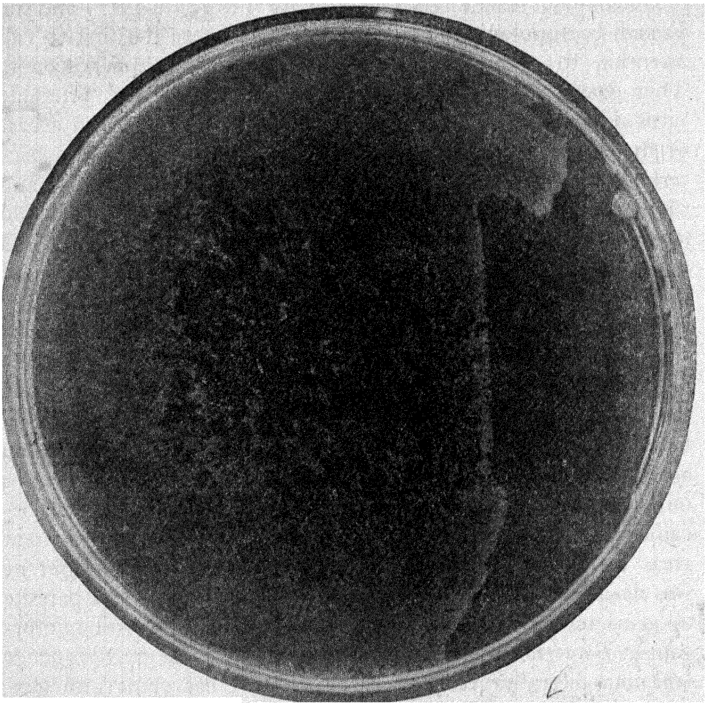


FIG. 26. An agar plate culture showing the effect of bacteriophage. The bacteria have grown as a continuous film over the surface of the agar (grayish white in the photograph), and the irregular dark areas are the places where the growth has been "eaten away" by a bacteriophage. (Photo reproduced through the courtesy of Dr. Alvin Wells.)

Modern research has shown clearly the close similarity of the bacteriophages to the recognized filtrable viruses we have discussed earlier in this chapter. Like the viruses, the bacteriophages appear to be extremely minute, filtrable bodies, invisible by ordinary methods of microscopic examination, not cultivable on lifeless media, and increasing only in the presence of the particular living, growing bacterial cells which are susceptible to their action.

There is evidently not one bacteriophage, but a number of different phages. Some have been shown to have a particle size of about 50 millimicron, while others have been found to be smaller (Fig. 25).

Nature of the bacteriophages. Scientists are not agreed as to the nature of the bacteriophages. D'Herrelle has continued to insist that the phages are living, ultramicroscopic organisms that prey upon bacteria, causing them to be destroyed. Topley and Wilson are inclined to agree with this view to the extent that they regard the bacteriophages as in all probability independent beings, not essentially different from the viruses which affect the cells of higher organisms and just as likely to be living entities as the yellow fever virus, for example. Many American authorities, however, are impressed with the evidence brought by the work of Bronfenbrenner, Krueger, Northrop, and others, which indicates that the phages are not living at all, but are inanimate, self-reproducing proteins in the nature of enzymes. Phages certainly increase in amount during active multiplication of the susceptible bacterial cells prior to lysis, and it is thought that the phage may be a product of the abnormal metabolism of those same bacteria. Motion picture studies of bacterial cultures under the influence of phage have shown that the bacteria at first grow and multiply with unusual rapidity and all their vital activities appear to be greatly exaggerated. Later, in the case of most species, the bacterial cells absorb water and become greatly swollen. Then, when the concentration of phage has reached a certain level, the cells burst and are destroyed. Just how a bacteriophage acts to start this destructive process is unknown. The true explanation of this remarkable phenomenon and an understanding of the real nature of the bacteriophages must await the results of future research.

Use of phages in treatment of disease. If bacteriophages destroyed bacteria as readily in the body as they do in a test tube they would have a very important influence upon the course of germ diseases. Some have claimed that it is indeed the activity of bacteriophages which largely determines whether we die or recover. But experimental studies have not borne out this claim and it is probable that phages have no appreciable effect upon the outcome of infections. Preparations containing active phages have been used to treat certain germ diseases for many years, but their value is very doubtful. Such beneficial results as have been reported are probably not due to any direct action of the phage itself, but to the fact that the phage-containing filtrates, which always contain some dissolved bacterial substance, are good vaccines, stimulating resistance to the particular germ involved.

REVIEW QUESTIONS—CHAPTER VII

1. What does the word *spirochete* mean? How are spirochetes related to other microbes?
2. Describe the four principal groups of spirochetes, and their distribution, and name the genera in which they are placed.
3. Describe the general morphology of the parasitic spirochetes. What is the best way to examine spirochetes microscopically? How may they be stained?
4. What types of spirochetes are easy to cultivate, difficult to cultivate? How do the *Treponema* differ in their requirements? How do spirochetes reproduce?
5. Are they resistant outside of the body? What peculiar susceptibility do they have which has made possible the successful chemical treatment of syphilis?
6. Name and describe briefly four varieties of *Treponema* found in the normal mouth and one found on the genitalia.
7. Into what three categories may the important pathogenic spirochetes be grouped?
8. Name and describe the causative organism, and discuss the occurrence and spread of infectious jaundice (Weil's disease).
9. Characterize briefly the disease called relapsing fever. Name and describe the organisms responsible for the European and African types of relapsing fever. How are the germs carried from person to person?
10. Name the causative organism of syphilis and of yaws.
11. When and by whom were the principal pathogenic rickettsiae recognized? What is the origin of the name *Rickettsia?*
12. Give the properties which distinguish the rickettsiae as a group.
13. What facts suggest that the rickettsiae were originally insect parasites only? What are the insect vectors involved in the principal rickettsia diseases? Define *European typhus*, *endemic typhus*, *murine typhus.*
14. What is meant by *intracellular parasitism?*
15. How may rickettsiae be cultivated in the laboratory? What is the chorio-allantoic membrane of a chick embryo, the tunica vaginalis?
16. What four human diseases are attributed to infection with rickettsiae?
17. Name the species responsible for typhus and for Rocky Mountain spotted fever.

18. Explain how the mosaic disease of tobacco plants was shown to be caused by a filtrable infectious agent. What was the first animal disease found to be caused by a filtrable virus?
19. Name some of the filtrable virus diseases of plants, insects, animals, and men. Are bacteria affected by viruses?
20. Are the individual viruses distinct entities?
21. Outline the basic properties of viruses and give a general definition.
22. Explain the general procedure followed in isolating and identifying a virus. Is it necessary to show that an infectious agent is filtrable before accepting it as a virus?
23. How is the identity of a virus proved by protection tests?
24. Describe how viruses may be cultivated artificially.
25. Explain the behavior of viruses in different hosts. Give examples of the change in virulence that may occur, when viruses are adapted to new hosts.
26. Discuss the infectivity and antigenicity of viruses. How do these properties explain some outstanding features of virus infections?
27. Discuss the importance and the probable nature of inclusion bodies.
28. What are elementary bodies? What are the properties of elementary bodies which indicate that they represent the actual, living virus? Have inclusion bodies and elementary bodies been found in all virus diseases?
29. What is the probable form in which all viruses exist? Give the approximate size in millimicra of the smaller, the medium sized, and the larger virus particles, and name viruses of each group. Compare the size of virus particles with that of the larger protein molecules and of rickettsiae.
30. What evidence has been found that certain viruses are identical with inanimate proteins? Mention a virus that has been isolated as an apparently pure protein. Discuss the importance of these findings.
31. Outline theories as to the nature and origin of the viruses.
32. Who discovered that bacteria may suffer a "filtrable virus disease," and when? What is the origin of the term *bacteriophage?* How did d'Herrelle demonstrate the action of a phage?
33. Outline the chief properties of bacteriophages. How do they resemble the filtrable viruses mentioned above?
34. Discuss the nature of bacteriophages. What usually happens to bacterial cells under the influence of phage?
35. What is the probable value of bacteriophages in the treatment of disease?

CHAPTER VIII

THE TRUE BACTERIA

THE FUNDAMENTAL properties of the true bacteria (*Eubacteriales*) which serve to differentiate them from related microbes described in previous chapters, may be summarized as follows: The true bacteria are extremely simple, microscopic single-celled plants, much simpler in structure and mode of development than the protozoa, yeasts or molds, or the spirochetes. Like other kinds of fungi, they possess no chlorophyll. Except in a very few species, the true bacteria do not have distinct nuclei, like the protozoa, yeasts and molds, but instead, the nuclear material is scattered throughout the cell. They reproduce by the process of simple transverse fission, which means a simple splitting of the cell across the short axis into two approximately equal parts. This method of reproduction is peculiar to the bacteria, for protozoa multiply in various complex ways, yeasts by budding, and molds by the formation of reproductive spores. The following is a satisfactory definition: *the true bacteria are microscopic, unicellular, chlorophyll-free, colorless plants, usually without a distinct nucleus, and reproducing by simple transverse fission.*

FORMS OF THE TRUE BACTERIA

Shape of individual cells. Among the true bacteria there are three basic forms (Fig. 27). The individual cells of some bacteria are *spherical* or nearly so, some are *cylindrical* or rod-shaped, and others have the form of a *curved* or *spiral rod.* The spherical forms are called *cocci* (singular, *coccus*), the cylindrical forms *bacilli* (singular, *bacillus*), and the spiral-shaped forms *spirilla* (singular, *spirillum*). Each one of the many kinds of the true bacteria is placed arbitrarily in one of these three morphological groups.

Cocci. The word *coccus* comes from a Greek word meaning "berry," and all of the cocci have a spherical or nearly spherical form like tiny berries. Many of the cocci are not perfectly round, but are flattened on one or more sides or are more or less elongated (Fig. 27: A, B, C, D, E, F). There are a great many species of cocci and they are very widely distributed. All are classified in the family *Coccaceae* (see diagram on page 46).

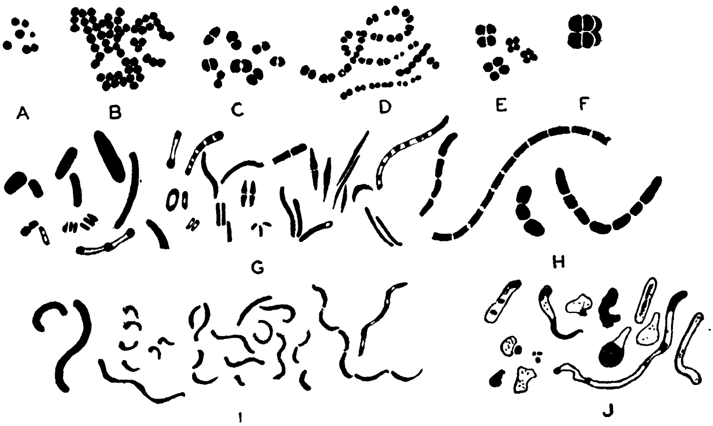


FIG. 27. Forms of the true bacteria. A, *single cocci;* B, *staphylococci;* C, *diplococci;* D, *streptococci;* E, *tetrads;* F, *sarcinae;* G, various forms of *bacilli;* H, *streptobacilli;* I, various forms of *spirilla;* J, involution forms.

Bacilli. In Latin, *bacillus* means "a little stick or rod," and the bacilli (Fig. 27: G, H) have the shape of little rods or cylinders. Individual kinds of bacilli show almost infinite variations of this basic shape. Some bacilli are so short and thick that they are nearly indistinguishable from cocci; others are long and slender. Their ends may be square cut, or rounded and more or less convex, or they may even be concave. One or both ends may be tapered to a blunt or a fine point. Most of the bacilli are straight, rigid rods, but some are slightly curved and less rigid. The bacilli, like the cocci, are widely distributed and very many species are known. The bacilli that form spores are placed in the family *Bacillaceae*, and the majority of those that do not in the family *Bacteriaceae*. The special family of bacilli called the *Nitrobacteriaceae* contains nonspore-bearing rods.

Spirilla. In this group are placed bacteria having the shape of a spiral, like a corkscrew. Some of the spirilla are short comma-shaped organisms; others are longer, more delicately coiled threads (Fig. 27: I). The body of the spirillum is rigid or nearly so. This distinguishes the spirilla from the *spirochetes.* The latter are more slender and flexible, difficult to grow in the laboratory and difficult to color with a stain, whereas the spirilla are comparatively short and thick and are as easily cultivated and stained as other forms of the true bacteria. There are fewer known species of spirilla than of cocci or bacilli. They are classed in the family *Spirillaceae.*

Cell groupings. Among the cocci, and to a lesser extent among the bacilli and spirilla also, the actively growing cells of some species tend to arrange themselves into characteristic groups. The character of the group is constant with any one kind of bacterium, therefore the grouping, as seen under the microscope, is of considerable help in the recognition of some species.

(*a*) *Groupings of cocci.* Practically all of the common cocci occur in groups of two or more cells, and cocci may be placed in one of the following classes according to the number and arrangement of the cocci:

Diplococci: cocci in pairs. Usually the adjacent sides of the paired organisms are flattened. The germs of meningitis, gonorrhea, and pneumonia, and many other familiar species of bacteria, have this form.

Streptococci: cocci in chains. When streptococci are multiplying, the cells always divide in the same plane of space and the new cells remain adherent, thus forming chains of the organisms resembling a string of beads. The length of the chain varies with the conditions under which the bacteria are growing, and also varies somewhat with the particular species of streptococcus. Several kinds of streptococci are found in connection with disease in human beings, but there are other streptococci that are entirely harmless.

Staphylococci: cocci in irregular, compact masses, resembling a cluster of grapes. These cocci divide in more than one plane of space and remain closely adherent, forming irregularly shaped masses. One can see in microscopic preparations of staphylococci that some of the cells lie behind others, just as some of the grapes forming a compact bunch will lie behind other grapes when the cluster is viewed from one side. There are many kinds of staphylococci, some of which cause disease.

Tetrads: cocci in groups of four. Only a few of the familiar species of bacteria show this arrangement. Sometimes the group of four cells is completely enclosed in a transparent envelope called a *capsule.* One of the best known of the cocci of this class is the *Micrococcus tetragenus,* an organism occasionally found in some disease conditions.

Sarcinae: cocci in cubical packets. The sarcinae are relatively large spherical cells which occur in compact masses, consisting of eight or sixteen individual organisms arranged in the form of a cube. The *Sarcina lutea* is a harmless bacterium of this class. It occurs commonly in the dust and can be cultivated readily from dusty air.

(*b*) *Groupings of bacilli and spirilla.* Some species of bacilli grow characteristically in pairs, and so may be called *diplobacilli.* Others may form long chains and are called *streptobacilli.* Some bacilli show a very striking *parallel* arrangement of the cells; others grow in a tangled intertwined mass.

The spirilla do not group themselves in any characteristic way, but they frequently grow in long, wavy chains. Often the individual spirilla making up the chain cannot be made out and the filament appears like a skein of twisted thread.

Variations from the typical form. In a laboratory culture of a single kind of organism the individual cells often vary considerably in size and shape and some may have far from the typical form. This is natural because all the individuals making up the population of the culture are not at the same stage of growth, and some may be degenerating, some may be dead. Very young cultures sometimes contain cells which look totally different from those in the same cultures a few hours later. Not only the age of the culture, but the character of the culture medium, the temperature of incubation, and other conditions affect more or less profoundly the shape and size of the organisms.

Involution forms. The most conspicuous changes in the shapes of bacteria are seen when cultures become old or when the organisms are growing in a medium which is for some reason unfavorable for their normal development. In these circumstances the organisms may appear swollen or shrunken and assume various bizarre shapes. They usually take stains very irregularly. Bacilli and spirilla often grow in long tangled filaments. Sometimes they merely lose their distinct outline and become granular. These changes are signs of a simple degeneration or involution. Cells showing these abnormal appearances as a result of age or unfavorable environment are referred to as *involution forms* (Fig. 27: J).

Pleomorphism. There are some species of bacteria which regularly show striking variations in the shapes of the individual cells in young and actively developing cultures as well as in old and degenerate ones. Such species are described as *pleomorphic* (many-formed). A good example is the bacillus of diphtheria (Fig. 69). The differences in size and shape shown by organisms of this kind are not due to the influence of age or unfavorable environment. Their meaning is not fully understood.

SIZE OF BACTERIA

Unit of measure; the micron. Micro-organisms are so minute that they cannot be measured accurately by any familiar scale. A special unit of measurement must be used. This is the *micron* (plural, *micra*). The Greek letter μ (pronounced "mu") is the symbol for a micron. 1μ is equal to *1/1,000 of a millimeter.* A millimeter is 1/1,000 of a meter. The *meter* (about 39 inches) is the standard unit for measuring length in the metric system—the system of weights and measures used in all scientific work (see Appendix A). In terms of inches, one millimeter is about 1/25 of an inch and a micron is equivalent to about 1/25,000 of an inch.

Actual size of some common species. The actual size of some of the best known germs is given in the following table. The size is determined quite accurately by special methods.

Name of Organism	*Actual Size of Individual Cells*
Staphylococcus aureus (boils, etc.)	0.6 to 1.0μ in diameter.
Streptococcus hemolyticus (sore throat, etc.)	0.4 to 0.75μ in diameter.
Pneumococcus (pneumonia)	0.8 x 1.2μ.
Gonococcus (gonorrhea)	0.8 x 0.6μ.
Meningococcus (meningitis)	0.8 x 0.6μ.
Corynebacterium diphtheriae (diphtheria)	Vary in length from 1.5μ to 6.5μ, in width 0.3 to 1μ.

Mycobacterium tuberculosis (tuberculosis)	2 to 4μ long, 0.3–0.5μ wide.
Eberthella typhi (typhoid fever)	1 to 3μ long, 0.8–1μ wide.
Clostridium tetani (tetanus)	2 to 4μ long, 0.3–0.5μ wide.
Vibrio cholerae (cholera)	1 to 2μ long, about 0.4μ wide.
Treponema pallidum (syphilis)	8–14μ long, about 0.2μ wide.

Comparisons with familiar objects. It is very difficult to conceive what these figures really mean. As a matter of fact, it is impossible to visualize accurately how extraordinarily tiny these germs are. It may help to realize that as we look at them through the microscope, we see them magnified about a thousand times. Perhaps the most helpful object for comparison is a good rule marked off in millimeters. Most of the bacilli are about 1μ (1/1000 of a millimeter) wide. This means that *1,000 of them could be laid side by side very comfortably across a millimeter space.* It would take more than 1,500 of them to span $\frac{1}{16}$ of an inch.

STRUCTURE OF BACTERIAL CELLS

Cell contents. When bacteria are examined in the living state in a drop of water under the microscope they appear as tiny, transparent, colorless masses in the shape of balls, cylinders, or spirals. They are only slightly denser than the water in which they are suspended and, therefore, they are hard to see. The interior of the cells is in most cases entirely homogeneous and appears to be made up of perfectly clear protoplasm. There is no head and no tail, and there are no special organs within the cell.

When bacteria are dried upon a glass slide and colored with a stain they usually show the same homogeneous appearance as when unstained. In most species the entire cell takes a solid deep color, like the nucleus of an animal cell.

Cell wall. Each organism has a cell wall or membrane which maintains the shape of the cell. But this membrane is so thin that it cannot be seen ordinarily in either stained or unstained preparations. Bacteria differ from yeasts and other plant cells which often have thick walls.

Capsules. Surrounding many bacterial cells there is a kind of gelatinous envelope or capsule. This is too thin to be seen in the case of most species. But in a few kinds the capsule is very well developed. It may be four or five times as wide as the bacterial cell itself. It does not take stains readily and when a comparatively weak dye, such as methylene blue, is used, the body of the organism may stain well but the capsule remains uncolored and appears as a clear zone about the cell (Fig. 28). It is possible to stain the capsule itself by special methods (Appendix B).

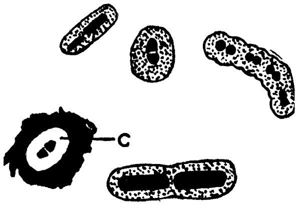


FIG. 28. Capsulated bacteria. C, appearance of the capsule in smears prepared by Gin's method (India ink smears).

Among the best known disease germs which develop large capsules are the *Diplococcus pneumoniae* (cause of lobar pneumonia), *Klebsiella pneumoniae* and related species (found in some cases of pneumonia and other inflammations), and *Clostridium welchii* (germ causing gas gangrene of wounds).

Probably all pathogenic bacteria develop at least a small amount of capsular substance when growing in the body tissues, and the most conspicuous capsules are seen on organisms freshly obtained from the living host. The capsules seem to act as a kind of defense against the germ-killing factors in body fluids which might otherwise overwhelm the organism. They are lost by most species after cultivation for some time in the laboratory, and accompanying the disappearance of the capsules there is usually a marked loss in capacity to produce disease.

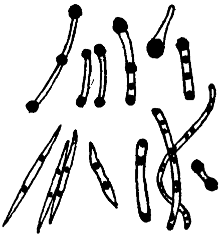


FIG. 29. Bacilli showing metachromatic granules.

Metachromatic granules. Some of the most important germs are distinguished by having somewhat more complicated internal structures than most other bacteria. The diphtheria bacilli, fusiform bacilli, the germ of glanders, and others, contain characteristic internal granules. These granules are called *metachromatic* (that is, change color) because when stained they take a tint different from that of the stain itself. Usually they color much more deeply than the remainder of the bacterial cell. In some species they occupy characteristic positions within the cells (Fig. 29). When situated at the extreme ends of a bacillus they are called *polar granules.*

MOTILITY OF BACTERIA

Flagella. All of the known spirilla and about half of the more familiar bacilli, but none of the ordinary cocci, possess the power of locomotion through liquids, that is, they are able to move independently from place to place. Bacteria that possess this capacity are said to be *motile;* those kinds that cannot move about independently are *nonmotile.*

The movement of motile bacteria can be plainly seen when the living organisms are examined under the microscope. Some kinds travel sluggishly; others with really amazing rapidity. It has been stated that the most active bacteria move about one 12/1,000 of an inch a second. If a man should move as rapidly in proportion to his size, he would be traveling somewhat more than a mile a minute. Sometimes active organisms pass across a microscopic field more rapidly than the eye can follow. It should be remembered, however, when examining motile organisms, that their apparent speed is magnified by the microscope in the same proportion as their size. If the bacteria are magnified a thousand times their movement also appears a thousand times faster than it really is.

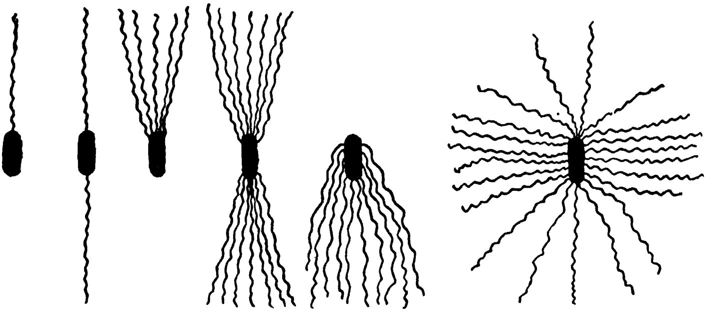


FIG 30. Showing the various arrangements of the flagella in different kinds of motile bacteria.

The organs of locomotion are extremely delicate, hair-like processes which extend out from one or more parts of the cell-bodies of the motile bacteria. These are called *flagella* ("little whips"). The rhythmic whipping motion of the flagella propel the organisms along. A bacterium may have one, a few, or many flagella, and these may arise either from one end, or both ends, or from all parts of the cell (Fig. 30).

Flagella are remarkably thin and delicate structures, having a diameter of only about 0.1 microns. However, they are often very much longer than the cell from which they arise. In unstained preparations flagella are quite invisible (although the movement they bring about is apparent). *They are also invisible in ordinary stained preparations,* therefore, one cannot tell by examination of a bacterium colored in the ordinary way whether it possesses flagella or not. It is possible to stain the flagella, however, by special methods. (Fig. 31. See also Appendix B.)

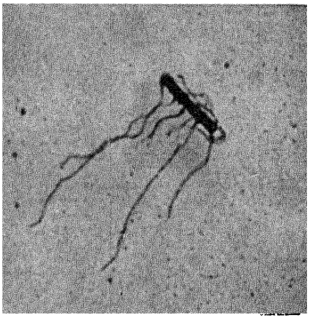


FIG. 31. The typhoid bacillus stained to show the flagella.

True motility and Brownian movement. The independent movement of motile bacteria from place to place by means of their flagella is spoken of as true motility. When bacteria are being tested for motility, this true locomotion must be distinguished from the movement produced by currents in the liquid which cause the organisms to drift about. And it is especially important that true motility be carefully distinguished from the mere vibration of the organisms in the fluid—a kind of motion known as *Brownian movement.* This is a physical phenomenon due to the rapid vibration of the molecules of the liquid itself which imparts an irregular bobbing or vibratory motion to any very small particles suspended in it. Nonmotile as well as motile bacteria, and in fact a particle of matter of any kind sufficiently small, will show Brownian movement when suspended in water. This motion is not progressive. A nonmotile organism will be impelled a little forward, then a little back, now a little this way, and now that, but it will never change its position with relation to neighboring organisms, which are likewise bobbing around like buoys in a heavy sea. On the other hand, a motile organism propelled by its flagella will be continually changing its position with respect to its neighbors.

SPORE-FORMATION BY BACTERIA

Certain kinds of *bacilli* are capable of changing into resistant bodies called **spores,** when conditions become unfavorable for their growth and multiplication. *Each individual bacillus becomes converted into a single spore.* This spore is able to withstand comparatively high temperatures and other unfavorable influences, and so serves to keep the organism alive when it would otherwise perish. If favorable conditions are again supplied, the spore returns to the original bacillus form. This remarkable property of spore formation is confined to a few species of bacilli only, but it has great practical importance.

Conditions under which spores form. Some of the spore-bearing species of bacilli change to the spore form more readily than others, but in any case the principal stimulus to spore-formation seems to be an accumulation of waste products resulting from the growth of the organisms themselves. The purpose of spore formation is, clearly, to protect the organism from unfavorable environmental influences.

Only one spore is formed from a single bacterium. Therefore, *spore formation among the bacteria is not a method of multiplication.* The molds, on the contrary, multiply by forming many similar bodies, also called spores. (The student should distinguish carefully between *mold* spores and *bacterial* spores.)

Process of spore-formation. The process of spore-formation consists in a kind of condensation of the cell contents into a round or oval body with a thick wall. The actual manner of spore-formation varies in minor details in different species of the spore-bearing bacilli, but the process is essentially the same in all cases. There appears first a spot, near the center or the end of the rod, which stains more intensely than the rest of the cell. This spot enlarges until in many cases it is of greater diameter than the cell from which it is forming. Very soon the center of this spot, instead of staining more intensely, ceases to stain at all when colored by ordinary methods. This is because the developing spore has now elaborated its dense outer wall, through which the stain does not penetrate. The wall itself may take a pale color, but the center of the spore does not stain, and appears as a bright, colorless spot. Meanwhile the remainder of the original cell decreases in size, stains very faintly, and gives every evidence of having given up its vital matter to the spore. Finally the remnant of the original cell disintegrates and the spore becomes free (Fig. 32: A).

A bacillus in its original active, growing state is referred to as the *vegetative form* of the organism. A bacillus in the process of developing its spore is said to be in the *sporulating form.* A completely formed spore detached from the bacterium in which it developed is called a *free spore.*

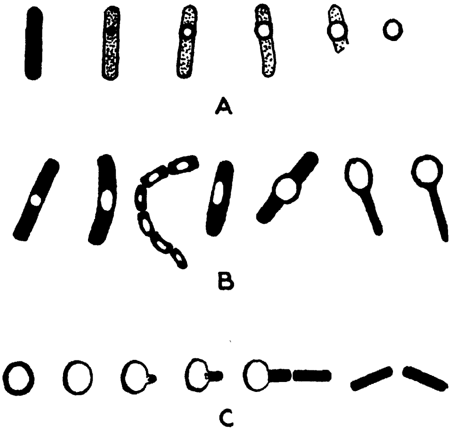


FIG. 32. Bacterial spores. A, stages in the formation of a spore. On the left is the vegetative form of the bacillus, on the right the free spore formed from it. B, the sporulating forms of different kinds of spore-bearing bacteria, showing characteristic differences in the size, shape, and position of the developing spore. C, one common method of germination of a spore back to the vegetative form.

Properties of free spores. A free bacterial spore has two principal properties, in both of which it resembles a seed: (1) *it is highly resistant* to physical and chemical changes which would injure or destroy the original organism, and (2) *it is capable of germination,* that is, it is able to return to the original growing vegetative form, *with all original properties intact,* when placed once more in a favorable environment.

Resistance of spores. The vegetative forms of spore-bearing bacteria are no more resistant than nonspore-forming organisms, but the spores resist drying, heat, and injurious chemicals to a remarkable degree. Spores of the anthrax bacillus, for example, dried upon a piece of thread, have been found capable of germination after a lapse of as much as thirty-five years. Furthermore, the bacilli germinated from these very old spores were still capable of causing anthrax in laboratory animals. The vegetative forms of most bacteria die in a few minutes when exposed to a temperature of 65°–70° C. (150°–160° F.), but most spores resist the action of live steam at 100° C. (212° F.) for a half hour or more. Dilute solutions of carbolic acid and other chemicals kill vegetative forms easily, but spores often withstand prolonged exposure to concentrated solutions of these substances.

Germination of spores. The process of spore germination is a reversal of the process of spore-formation. The details vary with individual species of bacilli, but in every case there is a rupture or an absorption of the dense outer wall of the spore and a return to the shape and size of the original vegetative form (Fig. 32: C). The most remarkable feature of this metamorphosis is the fact that the germinated bacilli possess, sometimes even in increased intensity, all the properties of the original organism.

Practical importance of spore-formation. Spore-formation is a property of a relatively small group of bacilli, the members of which have several characteristics in common. All the spore-forming bacilli can be found *in the soil,* where they exist in the spore state. Many of these organisms are native to the soil, and others are normal inhabitants of the intestinal tract of human beings and animals, and reach the soil through the intestinal discharges. Some kinds are always present in dust, and thus become widely distributed.

Fortunately only a few of these spore-bearing organisms are able to cause disease. Most of them are perfectly harmless saprophytes. The most important spore-forming bacilli which may produce disease are *Clostridium tetani,* the germ of tetanus, *Clostridium welchii,* the germ of gas gangrene, and *Bacillus anthracis,* the cause of anthrax.

The existence of spore-bearing bacteria must be taken into account in connection with practical methods used to destroy germs. When it is desired to render an object *sterile, i.e.,* entirely free of living organisms, the great resistance of the spores that may be present must be considered. The result is that the routine methods of sterilization are much more drastic than would be necessary if there were no spores to be destroyed.

REVIEW QUESTIONS—CHAPTER VIII

1. Summarize the fundamental properties of the true bacteria.
2. Give a definition of the *Eubacteriales*.
3. Name (singular and plural) the three morphological groups of the true bacteria. What is the basic shape of the organisms in each group?
4. Define *coccus, diplococci, streptococci, staphylococci, tetrads, sarcinae*. Describe and sketch some characteristic shapes and groupings of cocci.
5. Define *bacillus, diplobacilli, streptobacilli.* Describe and sketch shapes and groupings of bacilli.
6. Describe and sketch common forms of spirilla. How do spirilla differ from spirochetes?
7. Define *involution forms, pleomorphic.* Give an example of a pleomorphic organism.
8. Define *micron, millimeter, meter.* What is the symbol for a micron? What is the equivalent of a micron in inches?
9. Give the approximate sizes in micra of some important disease germs. Give an example to illustrate the extreme minuteness of bacterial cells.
10. Describe the cell-contents and cell-wall of bacteria as they appear unstained and stained.
11. Define *capsule.* How does the capsule appear in ordinary stained preparations? Name three species of capsulated bacteria.
12. Under what conditions are capsules best developed? What usually happens in laboratory cultures? What is the relation between capsule formation and the disease-producing power of capsulated organisms?
13. Define *metachromatic granules, polar granules.* Describe and sketch some bacteria which show internal granules.
14. Define *motile bacteria, nonmotile bacteria, flagella, true motility, Brownian movement.*
15. How can the movement of bacteria be observed? How fast can they travel? Describe flagella and tell how they are attached to the cell in different bacteria.
16. How can true motility be distinguished from Brownian movement?
17. Explain briefly what is meant by spore-formation in bacteria. Under what conditions do spores form? What is the purpose of spore-formation? Is spore-formation a method of reproduction for the bacteria?
18. Define a *bacterial spore, vegetative form, sporulating form, free spore.*
19. Describe the process of spore-formation.
20. What are the two principal properties of free spores? Give examples of the high resistance of spores.

21. What happens when spores germinate? What conditions are necessary for the germination of spores?
22. What kinds of bacteria form spores? Where are spore-bearing organisms found?
23. Name three important pathogenic bacteria which are spore-bearing.
24. How does the existence of bacterial spores affect our methods of sterilization?

CHAPTER IX

THE TRUE BACTERIA (Continued)

AMONG THE BACTERIA, as among other kinds of microbes, there is one group of organisms, the members of which use *lifeless matter for food.* These are *saprophytic bacteria.* Most of these are hardy species, able to grow in various places so long as they are supplied with nonliving matter as food. *Very few of them, however, are able to survive within the living body.* The great majority are, therefore, entirely harmless. The *natural habitat* of most saprophytes—*i.e.*, the environment in which they are naturally and constantly found—is in some part of the outdoor world, as in the soil, or water. We find some species adapted to a particular kind of soil, or to the water of a certain type of spring, or to some other particular place. Many saprophytic organisms grow naturally in the intestinal contents of animals and human beings.

In contrast with the saprophytic types, there is a large group of *parasitic bacteria.* These organisms are accustomed to living on or in the bodies of living things from which they derive their nourishment. *Most of them die very quickly when cast off from the body.* The natural habitat of parasites, therefore, is some part of the surfaces or tissues of living plants, animals, or human beings. The various parasitic organisms are usually adapted to existence in a limited area of the body only. Some species are found constantly in the mouth, for example, but not elsewhere. The disease-producing parasites are still more closely confined to life in particular situations. They are able to cause disease in certain species only. Most of them enter the body tissues only by a certain path, and leave the body by a certain route; they multiply only in certain organs, and poison only certain tissues.

As a consequence of this distribution of the various kinds of bacteria in certain particular regions, the same places are always inhabited by about the same kinds of bacteria. The usual varieties of micro-organisms naturally and constantly present in a particular locality are said to constitute the *normal flora* of that region. *Escherichia coli,* for example, is constantly found in the intestinal tract of human beings. The intestine of man is, therefore, its natural habitat, and this organism is one of the species of bacteria comprising the normal flora of the intestine.

Food requirements. The different kinds of bacteria use a great variety of natural substances as food. A few exceptional species are able to utilize simple *inorganic* substances as the sole source of nourishment, and cannot use more complex materials. These are called *autotrophic* bacteria. An example of such organisms is the so-called nitrifying bacteria (family *Nitrobacteriaceae*) which can build up nitrates from ammonia and nitrites. These organisms are normal inhabitants of the soil, and have a very important influence on its fertility (Chapter X).

The great majority of bacteria are quite unable to utilize inorganic materials, but must derive their nourishment from *complex organic substances.* In distinction from the autotrophic forms these are called *heterotrophic* bacteria. Saprophytic bacteria require their food in the form of lifeless animal or vegetable matter. The parasitic bacteria, on the other hand, are adapted to the use of organic food derived directly from the living tissues of plants, animals, or human beings.

For growing most bacteria in the laboratory, rich food mixtures are used. The commonest culture media contains an extract or an infusion of meat, and also partially digested protein substances in a readily available form. Other materials used are egg, milk, cooked meat, and coagulated blood serum. Often glucose (grape sugar), or other carbohydrate, is added to furnish a ready source of energy. Even such rich food does not suffice for the cultivation of many parasitic bacteria, especially certain delicate pathogenic organisms, unless some fluid freshly obtained from a living body, such as blood serum or whole blood, is added to the medium.

Reproduction. *Method of reproduction.* Among the true bacteria, reproduction is entirely an *asexual* process, that is, there are no male and female cells, and no fertilization is needed. After the parent cell has attained its maximum size, it splits across the short axis to form two new cells. This is the process of *simple* *transverse fission.* The new individuals grow until they are of maximum size, and if conditions are still favorable, they each split into two new cells as their parent did. The bacteria are potentially immortal. The daughter cells resemble the parent cells because they are literally parts of them.

The actual process of cell division is illustrated in Fig. 33. A slight cleft or *fissure* develops across the short axis, this deepens and soon becomes a complete separation. New cell walls are formed at the point of division and the two new cells, each of almost the same size, are now independent individuals. They may remain close together, as happens often with the cocci, or they may separate entirely, according to the species.

Rate of multiplication. The rate at which cell division occurs varies with the species and with the conditions in which the organism is growing. There are a few slow growing kinds, but most species are able to multiply with great rapidity. When conditions are the best, less than half an hour may represent the span of a whole generation. In other words, the time necessary for many species to reach maturity and divide into two new cells may be only about one half-hour. This means that, *starting with just one organism* of this kind at 9 o'clock, by noon there would be only 64, but by 9 o'clock the same night there would be very nearly seventeen million (17,000,000), and in twenty-four hours the theoretical progeny would number about two hundred and eighty trillion (280,000,000,000,000)!

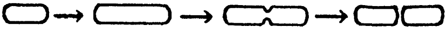


FIG. 33. Diagram illustrating the process of reproduction in the true bacteria. This method of multiplication is called simple transverse fission, and the bacteria are named *Schizomycetes* (the fission-fungi).

This enormous total resulting from the multiplication of a single original organism is possible only in theory. It could not happen unless the half-hour division rate continued throughout the twenty-four hours and this never occurs under any circumstances. After a very short period at the maximum rate, many factors combine to check the rate of reproduction, especially the decreasing availability of food and the accumulation of waste products. Despite these natural checks, the capacity of most bacteria to increase in number so rapidly, during the limited time in which conditions remain favorable, constitutes one of their most important properties and in large measure explains their great power.

INFLUENCE OF ENVIRONMENT UPON THE GROWTH OF BACTERIA

Bacteria are literally at the mercy of their environment, and may be unfavorably affected by very slight changes in any one of the physical or chemical features of their surroundings. Life is possible for them only within definite and rather narrow limits set by the nature of the environment, and they are most active within still narrower limits. The spore-forming types are the only kinds that have any protection against unfavorable conditions. This is fortunate from a practical point of view, for it means that by controlling environmental conditions we can stimulate bacteria to grow, or stop their growth, or destroy them, as we wish.

The factors of the environment which most directly affect the life of bacteria are: (1) moisture, (2) light, (3) temperature, (4) osmotic pressure, (5) reaction, (6) oxygen supply, and (7) the character of neighboring microbes.

Moisture. An abundance of water is just as essential for the growth of bacteria as an adequate supply of nourishing food. In fact, bacteria cannot be nourished without water, because food elements must be in solution before they can be absorbed through the cell walls of the organism. All kinds of bacteria grow best in a very watery medium and in an atmosphere saturated with moisture. A total lack of moisture prevents growth, and, therefore, bacteria do not develop on dry objects and such objects do not decompose.

Light. Bacteria differ sharply from green plants in their reaction to light. The chlorophyll in the leaves of green plants helps to nourish the plant in the presence of sunlight, and the development of green plants is in direct proportion to the hours of sunlight they enjoy. The bacteria, however, have no chlorophyll, and their growth is not aided by sunlight. On the contrary, most species are injured by even diffuse daylight and are killed in a few hours by direct exposure to the sun. It is the ultraviolet rays of the sunlight which have this destructive effect.

Temperature. *Optimum growth temperatures.* For each species of bacteria there is a temperature at which the organism is in every way most active, and at which the most rapid growth and multiplication take place. This is called the *optimum growth temperature.* This always corresponds to the usual temperature of the natural habitat of the species. For the great majority of the saprophytic organisms inhabiting the soil and other places outside of living bodies the optimum growth temperature is 25°–30° C., that is, just a little higher than the usual room temperature. There are some species found in the soil, in hot springs, and in intestinal contents of animals whose optimum temperature may be as high as 60° C. (140° F.) or higher. These are called *thermophilic* (heat-loving) *bacteria.* At the other extreme, there are a few exceptional species which grow best at temperatures only slightly above the freezing point. These are called *psychrophilic* (cold-loving) *bacteria.*

The optimum temperature for the bacteria parasitic on the body of human beings or other warm-blooded creatures is the temperature normal to the body, *i.e.*, 37° C. (98.6° F.). Laboratory incubators in which cultures of disease germs from the human body are grown are adjusted to keep a constant temperature of 37° C. Bacteria which are parasitic on the bodies of *birds* grow best at a higher temperature, 41°–45° C., because this is the normal temperature of birds.

Minimum and maximum growth temperatures. At temperatures above or below the optimum, organisms are less active, and grow and multiply more slowly. For each species there is a definite limit in each direction beyond which growth will not occur. The lowest temperature at which growth is possible is called the *minimum growth temperature*, and the highest, the *maximum growth temperature.* The minimum temperature for most of the organisms living on the human body is about 20° C., and the maximum is about 42°–45° C. Some of the pathogenic germs are so closely adjusted to the body temperature (37° C.) that they multiply scarcely at all at any other; most of them, however, will grow between 36° C. and 42° C.

Effect of low temperatures. At temperatures below the minimum growth temperature bacteria cease to develop, but most species are not killed by cold. Some organisms will withstand a short exposure to a temperature far below that of the freezing point of water, and almost all species will remain alive for weeks at temperatures just above the freezing point. But growth of the ordinary bacteria does not occur in the cold, and the organisms will not multiply until returned to a higher temperature approximating that of their natural habitat. Sudden changes of temperature are more harmful than abnormally low or moderately high temperatures.

Effect of high temperatures. Thermal death point. High temperatures are much more injurious than low temperatures. Sometimes an organism will continue to grow at an abnormally high temperature, but in doing so it will lose some of its characteristic properties. The anthrax bacillus, for instance, when cultivated for some weeks at 42° C., instead of 37° C., loses its capacity to form spores and some of its disease-producing power.

If any bacterium or spore is heated above the maximum growth temperature to a sufficient degree, it will be killed. The lowest temperature at which an organism is killed by heat is called the *thermal death point.* There is much variation in the thermal death point of different species, that is to say, some are more quickly killed by abnormally high temperatures than others. It is very important to remember, however, that *all nonspore-forming disease-producing bacteria, and almost all other nonspore-forming organisms, when exposed in watery liquids to a temperature of 60°–65° C. (140°–150° F.) are killed within one half hour.* This fact is the basis for the method of rendering milk free of disease germs by *pasteurization.*

Osmotic pressure. When two solutions of *different concentration*—as, for example, a solution containing *1* per cent of salt, and another containing *25%* of salt—are separated by a thin membrane made of parchment or similar material there will be a passage of water and of salt through the membrane until the concentration of salt on each side of the membrane becomes approximately the same. (Fig. 34). The passage of water or of dissolved substances through a membrane is called *osmosis.* If the membrane will allow only water to pass through it is said to be *semipermeable.* The greater the difference in concentration of the solutions on either side of the membrane, the greater is the *osmotic pressure.*

The phenomenon of osmosis is of fundamental importance, because *the cell walls of living cells, including bacteria, act like* *semipermeable membranes.* Water passes freely into or out of bacterial cells, according to whether the organisms are in a more dilute, or a more concentrated solution than exists within the bodies of the bacteria themselves. Their protoplasm always contains more dissolved substances than pure water, so that if

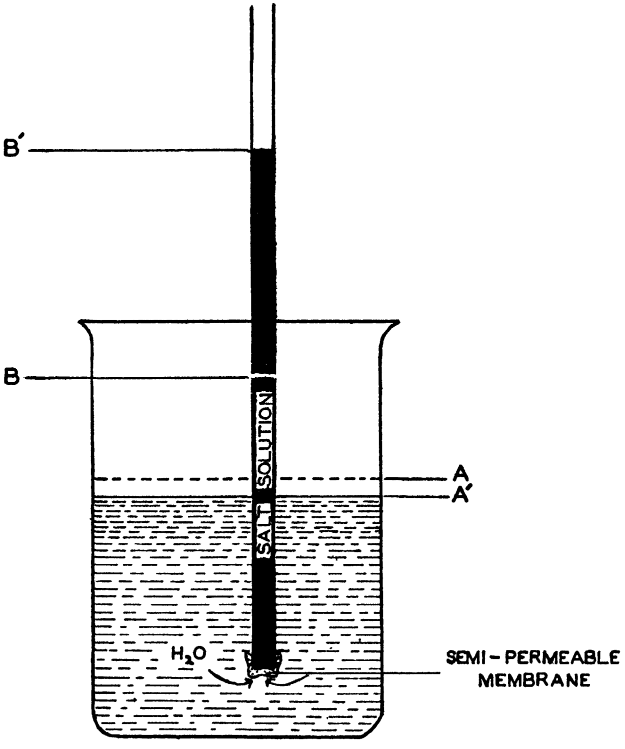


FIG. 34. Osmosis. A piece of goldbeater's skin, which acts as a semipermeable membrane, has been tied about the end of a glass tube, the tube has been partly filled with a strong salt solution and immersed in a beaker of water. Water passes through the membrane into the salt solution by *osmosis,* thus increasing the volume of the solution within the tube. A, original level of water, A′ final level of water, B, original level of salt solution, B′, final level of salt solution. If the salt solution were originally in the beaker and the water in the tube, the water would pass by osmosis *out* of the tube. The cell walls of bacteria and other living cells act like semipermeable membranes.

bacteria are suspended in distilled water, this water will diffuse *into* the bacterial cells, causing them to swell and perhaps to burst. When a cell is disrupted in this manner, the process is spoken of as *plasmoptysis.* On the other hand, if bacteria are placed in a very concentrated solution, such as a strong brine, water will pass *out* of the bacterial cells into this solution, so that the organisms will shrink and die. This is called *plasmolysis.*

In all biological studies wide use is made of so-called normal salt solution, or physiological salt solution, made by dissolving 0.85 per cent sodium chloride in water. This solution has a salt content equal to that of normal blood, and consequently red or white blood cells, other tissue cells, and also bacteria and other microbes, maintain their structure intact when suspended in it. It is a so-called *isotonic* solution. A solution containing a *lower* concentration of salt, such as water, is said to be *hypotonic,* and one having a *greater* proportion of salt, such as brine, is said to be *hypertonic.*

The effect of concentrated (hypertonic) solutions in destroying bacteria is of practical importance. Every one is familiar with the practice of preserving meat in concentrated brine (salt) solution. The meat is preserved because bacteria which would spoil it cannot live in the brine. Similarly, fruits or other perishable foods, may be preserved in strong sugar solutions. We can see why sweetened condensed milk, or thick sugary syrups, keep so well.

In order for bacteria to develop, their food must be supplied in diluted form. They grow best in watery solutions containing only a small percentage of sugar or other food substances, and particularly well if the proportion of dissolved substances is about the same in the food solution as within the bacterial cells.

Reaction. By *reaction* is meant the degree of acidity and alkalinity. Most bacteria are very sensitive to an excess of acid and grow best in a medium which is neutral or very slightly alkaline. When bacteria decompose sugars, the acids produced often accumulate to such a degree that they stop the growth, or cause the death of the organisms. Often chemical "buffers" are added to culture media to absorb acid as it is formed. Each species is nicely adapted to the particular reaction of its natural habitat. When bacteria are cultivated in the laboratory, we find it necessary to adjust the reaction of culture media with great care in order to meet the exacting requirements of many delicate species.

Oxygen supply; aerobic and anaerobic bacteria. Every living thing must have oxygen in some form. Most bacteria and other microbes derive their oxygen *from the air* as human beings do. These bacteria are called *aerobes.* Their activity is dependent upon an abundant supply of atmospheric oxygen.

There is, however, an important group of bacteria for which the oxygen of the air is actually a poison. These remarkable organisms cannot use gaseous oxygen or live in its presence. Bacteria of this group are called *anaerobes.* They must derive their oxygen from some *compound* which is rich in it, such as a sugar. *They will grow only where the air is totally excluded,* unless accompanied by aerobic organisms which use up the atmospheric oxygen. Special methods, designed to exclude atmospheric oxygen, must be used in order to cultivate anaerobes in the laboratory. (See Chapter XIV.) Among important pathogenic anaerobes are the germs of syphilis, tetanus, gas gangrene, and botulism.

Intermediate between the strict aerobes and the strict anaerobes are many species able to use either free or combined oxygen, although each species shows a decided preference for a particular degree of oxygen tension. Many pathogenic bacteria grow best in an atmosphere containing only a *low concentration* of free oxygen, and some are aided by the presence of an increased proportion of *carbon dioxide.*

Relation to other microbes. The life of any single organism in its natural habitat is profoundly affected by the presence of other microbes. In nature the various species are never found quite by themselves as we have them in our pure cultures in the laboratory. Instead, every particular place has its characteristic bacterial population, comprising a number of species, often of the most diverse types, living in intimate relationships with one another. For example, on the gum margins in the human mouth there is always to be found a certain group of organisms of several different species. These bacteria constitute the normal flora of the gum margin. They grow there constantly because they have become adapted to the natural conditions on the gums, and also because they have become adjusted to living together. There is, similarly, a characteristic normal flora on the skin, in the throat, at the various levels of the intestinal tract, etc., consisting in each case of several distinct species living, as it were, in a state of balance with environmental conditions and with one another.

Symbiosis. Often we find two organisms so adjusted to each other that they will grow only very poorly without their usual companion, and in some cases will not grow at all when separated. When organisms live together in such a way as to be mutually helpful, we say they are growing in *symbiosis.* For example, an anaerobic organism may grow symbiotically with an aerobic one, provided the latter is sufficiently active in using the atmospheric oxygen which would otherwise injure the anaerobe. Usually the symbiotic relationship is far more complicated than in this example, and is due to factors too subtle and too complex to be measured.

Mixtures of organisms sometimes accomplish what individual species are unable to do. The decomposition of dead plants and animals and other organic materials is never completely carried out by any one kind of bacterium, but by successive groups of organisms living more or less in symbiosis. A considerable number of human diseases, as, for example, inflammations of the appendix and other parts of the digestive tract, ulcerations in the mouth and throat, and wound infections, are usually caused by a mixture of organisms. Often the disease-producing power of a particular species is increased by the presence of certain other microbes.

Bacterial antagonism. On the other hand, the life of some organisms is *antagonistic* to the existence of others. The waste products, or the byproducts, of one organism may injure or kill a neighboring organism. In the natural souring of milk, a number of different species contribute to the production of the acid which clots the milk, but they are killed off one by one as the process proceeds, until in many instances the only species that survives when souring is complete is a certain streptococcus that is not injured by the high acid content of the fully curdled milk. Disease-producing germs may invade a particular spot, such as the tonsil, the eye, or the small intestine, and cause the temporary disappearance of almost all of the bacteria normally there. The bacteria that vigorously decompose sugars, and thereby produce considerable amounts of acid, are, as a general rule, antagonistic to those kinds most active in the decomposition of proteins, for the latter are easily injured by acid. By persistently drinking soured milk containing acid-tolerant, fermenting bacteria, it is possible to implant these organisms in the intestines, and if at the same time a large amount of carbohydrate is eaten, these bacteria will produce so much acid that the proteolytic types will be driven out.

SOME IMPORTANT DECOMPOSITION PROCESSES AND PRODUCTS RESULTING FROM BACTERIAL GROWTH

The real importance of bacteria lies in the chemical activity which accompanies their growth. The changes they cause in the materials on which they are feeding depend upon their ability to bring about certain definite chemical reactions. The bacteria are very active chemically. They are, in fact, master chemists, causing many remarkable transformations impossible to duplicate in a laboratory.

Their extraordinary chemical power is due to the fact that bacterial cells produce specific *enzymes,* or ferments, just as higher organisms do. Enzymes are those peculiar substances formed only by living cells, which act, each one in a specific way, to bring about the digestion of food and practically all the other complicated chemical reactions that go on in the living body. The various species of bacteria are able to bring about certain characteristic chemical changes because they elaborate enzymes with specific powers.

Putrefaction of proteins. The decomposition of protein substances by micro-organisms is called *putrefaction* or *proteolysis.*

Proteolytic bacteria. The kinds of bacteria which can bring about putrefaction of proteins are referred to as *proteolytic* (protein-dissolving or protein-splitting) *bacteria.* Of course, all bacteria can attack the simpler nitrogenous compounds to the degree necessary to obtain nitrogen for growth, but the species commonly called proteolytic are those which can *decompose complex native protein materials, such as meat, or egg, or blood-serum.* These are the organisms that bring about the foul smelling, rotting processes with which everyone is familiar—the decomposition of foods, of dead bodies, of feces, and of other lifeless matter. No one organism or group of organisms carries out the entire decomposition of complex organic matter, but rather, successive groups take part, one group commencing where another leaves off. *Anaerobic* saprophytic bacteria play a dominant part in the earlier stages of decomposition, when foul odors are produced, but in the final stages aerobic bacteria predominate.

Products of protein decomposition. The protein molecule is first split into what are called proteoses, then into simpler and simpler fractions—peptones, polypeptids, and amino-acids, which may finally be split to yield ammonia or free nitrogen. The decomposition does not necessarily occur in any regular order, and at the same time that more complex substances are being broken down, simpler compounds, such as carbon dioxide (CO_2), hydrogen (H), and water (H_2O), are continually being formed. The foul odors are due to the liberation of indol and skatol (the substances chiefly responsible for the characteristic odor of human feces), hydrogen sulphide (H_2S), and other malodorous compounds.

Some of the products formed when proteins are partially decomposed are poisonous. The most interesting of such products are the compounds called *ptomaines.* These substances are found in decomposing flesh, eggs, etc. Some ptomaines are slightly poisonous, others are not. At one time it was thought that ptomaines played an important part in human disease, but it is now known that this is not the case. They are still popularly regarded as the cause of so-called "ptomaine poisoning"—an acute intestinal disease which may follow the eating of a certain food,—but this idea is incorrect. This kind of poisoning is not due to ptomaines, but to the presence in the food of certain species of bacteria and poisons formed by them (Chapter XXXV).

Fermentation of carbohydrates. The decomposition of sugars, starch, and other carbohydrates is called *fermentation.* The power of micro-organisms, especially the yeasts, to bring about fermentation has much practical importance.

Fermentation by bacteria. The various species of bacteria differ sharply in their capacity to ferment particular carbohydrates. Some few species can decompose cellulose and starch, the most complicated substances of this kind. Most species can utilize only the simpler sugars, such as *dextrose* (grape sugar), *saccharose* (ordinary table sugar, cane sugar), *maltose* (malt sugar), or *lactose* (milk sugar).

Fermentation reactions furnish one of the most ready means of distinguishing one kind of bacterium from another. Thus *Escherichia coli*, a normal inhabitant of the human intestine, can be distinguished from *Eberthella typhi* (germ of typhoid fever), which it otherwise closely resembles, by the fact that it ferments lactose, while the typhoid bacillus does not. Similarly the individual members of other groups of closely related bacteria can be identified by sugar fermentation tests.

Products of fermentation. When carbohydrates are fermented by bacteria various organic *acids* are formed, and often *gases* are produced also. Sometimes only acid forms, in other cases both acid and gas.

One of the commonest acids produced is *lactic acid.* The so-called "lactic acid bacteria" cause the souring of milk and play an indispensable part in many commercial processes. *Acetic acid* is also commonly produced and *butyric acid* frequently results from anaerobic fermentation. A small amount of *alcohol* is produced during fermentation by some bacteria.

The gases most commonly formed from sugars by the few gas-forming bacteria studied in medical bacteriology are *carbon dioxide* (CO_2) and *hydrogen* (H). *Methane* or marsh gas (CH_4) is produced from starch and cellulose by saprophytic organisms and can be observed arising as bubbles to the surface of the water of sluggish streams, ponds, or swamps, containing decomposing vegetable matter.

Most of the bacteria that are active fermenters and produce large amounts of acid can withstand an exposure to a higher concentration of acid than other species. Such organisms are called *aciduric bacteria.* When they are growing in sour milk, for example, all the other types of organisms are eventually killed off as the milk gets more and more acid.

Decomposition of fats. Butter, lard, and other fatty substances do not decompose readily. A few species of bacteria, however, are able to break down fats slowly into glycerin and the particular fatty acids composing them. Butter may be made rancid by the growth of such organisms.

Production of pigments. A number of bacteria are able to manufacture colored substances. These organisms are called *chromogenic* (pigment-producing) bacteria. The pigments may have any of the familiar colors of the spectrum. Each species makes its own characteristic color. Some of the pigments are soluble in water and therefore become diffused throughout the culture medium. Others are soluble only in chloroform or similar solvents.

So far as we know, pigment production has no relation to any other of the significant properties of the organisms. Both pathogenic and nonpathogenic species, of all morphological types, are found among the chromogens. One of the saprophytic bacteria often present in the air of rooms is the *Serratia marcescens* (old name *Bacillus prodigiosus*), which produces a brilliant red color. The species of *staphylococci* common on the human skin develop orange and yellow pigments in laboratory cultures.

Pseudomonas aeruginosa (old name *Bacillus pyocyaneous*), an organism not infrequently found in the pus of long-standing wounds, discharging ears, and the like, develops bluish green pigments which impart a characteristic color to the dressings. Old cultures of *tuberculosis germs* have a brownish or yellowish color.

Production of toxins. An exceedingly important property of certain pathogenic bacteria is their ability to elaborate specific *toxins* (poisons). Almost all of the bacteria which cause disease in human beings are in some degree poisonous to the body. In many diseases the toxin of the germ does the chief damage. Bacterial toxins are of two kinds: (1) **exotoxins** and (2) **endotoxins.**

(1) *Exotoxins. An exotoxin is a poisonous substance which diffuses into the surrounding medium from the body of a living bacterium.* It is a product of the metabolism of the growing germ, and since it is soluble in water, it appears in solution in the medium in which the organisms are growing. If the culture is passed through a bacteriological filter a *sterile, germ-free filtrate is obtained which contains the toxin in solution.* This is the way an exotoxin is secured for study. It is nothing more than the sterile *filtrate* from a broth culture of the germs (Figs. 53 and 60).

The exotoxin formed by any one germ is *specific*, and differs from the exotoxin formed by any other germ. All the exotoxins, however, have certain fundamental properties in common which distinguish them from endotoxins, and from ordinary chemical poisons. As explained above, they are all products of living germs and can be separated by filtration from the organisms themselves. When introduced into the body of a susceptible person, an exotoxin will reproduce the characteristic symptoms and lesions of a natural infection with the germs from which the toxin originated. Thus, if the exotoxin of the diphtheria bacillus is injected into the body it will injure the tissues just as they are injured in the course of diphtheria. The poisonous effect of exotoxins is not manifested until a certain interval of time has passed after their introduction—the incubation period. This is in contrast to the chemical poisons, for example, strychnine, which act immediately. Finally, a most important property of all exotoxins is that, whenever they are introduced into the body, they stimulate the formation of specific *neutralizing antibodies* called *antitoxins.*

There are only a few pathogenic bacteria that develop powerful exotoxins. The most important are *Corynebacterium diphtheriae* (diphtheria), *Clostridium tetani* (tetanus), *Clostridium botulinum* (botulism), *Streptococcus scarlatinae* (scarlet fever). *Clostridium welchii* (gas gangrene), and *Shigella dysenteriae* (dysentery). Milder exotoxins are formed by a considerable number of other pathogenic species. Of special importance are the toxic products that act directly upon red blood cells (called *hemolysins*), and upon white blood cells (*leukocidins*). The special properties of these toxins and other active products of pathogenic organisms are described in later chapters.

(2) *Endotoxins. An endotoxin is a poisonous substance contained within the body of a bacterium which is released only after the organism dies and the cell is disintegrated.*

Endotoxins are not so well understood as the exotoxins. They are best regarded as *poisonous elements in the protein structure of the cells themselves,* which are liberated in soluble form only after partial decomposition of the cell-protein. When organisms of the endotoxin-producing type are grown in liquid media, they do *not* excrete toxin into the broth. But if the organisms themselves be collected and their bodies disintegrated in some manner, *the dead, disrupted cells can be shown to be poisonous.* Endotoxins are not specific for each kind of organism, but probably owe their toxic action to the same chemical grouping in all cases.

.Endotoxins differ sharply from exotoxins in that they do *not* stimulate the formation of antitoxins.

Practically all disease germs develop endotoxin in their bodies, and *this type of toxin is the only one produced by most species.*

The organisms of typhoid fever, dysentery, cholera, gonorrhea, and epidemic meningitis are examples of bacteria with considerable amounts of endotoxin.

PRINCIPAL GENERA OF THE TRUE BACTERIA AND SPECIES OF MEDICAL INTEREST

In the following paragraphs are listed the principal genera and species of bacteria not previously mentioned, with special attention to those of medical interest.

THE FAMILY COCCACEAE (THE COCCI)

Here are placed the bacteria whose cells are usually spherical. Division into genera is based principally upon the character of the cell-groups formed, whether pairs, chains, packets or irregular masses, and upon the Gram staining reaction. They are all typically Gram-positive except the *Neisseria.*

Neisseria. The organisms of this genus are parasitic, Gram-negative diplococci, the cells occurring in pairs, flattened on adjacent sides so that the pair has a sort of bisquit or coffee-bean shape.

Neisseria gonorrheae, the gonococcus, is the specific cause of gonorrhea.

Neisseria meningitidis, the meningococcus, causes epidemic cerebrospinal meningitis.

Neisseria catarrhalis is an ordinarily non pathogenic species found as a parasite in the normal mouth or respiratory tract.

Neisseria pharyngis and related forms represent a large group of nonpathogenic, Gram-negative diplococci which are constantly present in the normal mouth and throat. Many are chromogenic, forming greenish-yellow pigments.

Diplococcus. In this genus are Gram-positive cocci, occurring characteristically in pairs, and often capsulated.

Diplococcus pneumoniae, the pneumococcus, is the chief cause of lobar pneumonia. Unlike streptococci, this organism is soluble in bile.

Streptococcus. This genus contains those spherical bacteria which grow normally in chains. A primary division within this large and important group is made between the parasitic forms, many of which are highly pathogenic, and the purely saprophytic torms found in milk and other dairy products. Also the *anaero-*

bic stains form a separate subgroup. All the streptococci are customarily divided into three main groups on the basis of the appearance of their colonies on blood agar media. These groups are: (1) the *hemolytic* or "*beta type*" streptococci, whose colonies are surrounded by a wide clear zone in which the hemoglobin of the blood has been decolorized, (2) the *viridans* or "*alpha type*" streptococci, whose colonies are green and are surrounded by a zone of greenish coloration, and (3) the *anhemolytic*, *indifferent*, or "*gamma type*" streptococci, whose colonies produce no change in the blood agar.

Streptococcus pyogenes (or *Streptococcus hemolyticus*) is the name usually applied to the pathogenic, hemolytic streptococci so frequently encountered in a variety of infectious processes in man and animals.

Other species names have been given to the strains of hemolytic streptococci isolated from particular sources, for example, *Streptococcus scarlatineae*, *Streptococcus erysipelatis*, and *Streptococcus epidemicus* have been applied to the streptococci of scarlet fever, erysipelas, and septic sore throat, respectively.

Streptococcus viridans is most often used to designate the green colony streptococci. Various other names have been suggested for particular strains, but their validity is doubtful.

Streptococcus anhemolyticus is usually applied to the indifferent colony streptococci.

Streptococcus lactis is one of the best known species of the harmless, saprophytic, so-called lactic acid bacteria that are abundant in sour milk and similar substances.

Staphylococcus. The cocci of this genus are Gram-positive cells typically arranged in irregular, grapelike clusters.

Staphylococcus aureus is the most common cause of boils and similar infections; it forms a golden-orange pigment.

Staphylococcus albus develops a white growth on solid media; the pathogenic strains are less virulent as a rule than *aureus* strains.

Staphylococcus citreus is a saprophytic, nonpathogenic species, forming a lemon-yellow pigment.

Micrococcus. In this genus are placed a rather heterogeneous set of cocci, the majority of which are saprophytic organisms of the soil and water. They occur in irregular masses, pairs, or packets.

Micrococcus luteus is an example of a saprophytic species, forming a yellow pigment.

Micrococcus tetragenus is a species of medical importance.

It occurs as packets of four cells, surrounded by a capsule. It is not uncommon in the sputum of tuberculous patients and is at least potentially pathogenic.

Sarcina. These cocci, under favorable conditions, form regular cubical packets, consisting of eight cells each. There are many species, all saprophytes; most of them form abundant yellow-orange pigments.

Sarcina lutea is one of the best known species.

THE FAMILY BACTERIACEAE (NONSPORE-BEARING BACILLI)

This is the largest family of the true bacteria; it contains the greatest number of different genera and species. The nomenclature and classification within this large family is in a highly unsettled state at the present time. The names given here are those most widely accepted by American authorities.

Serratia. This genus name is applied to a very small, Gram-negative, motile bacillus, which forms a brilliant red pigment; it was named *Serratia marcescens* as early as 1823. This species is still widely known by its older name *Bacillus prodigiosus.* It is often found on foodstuffs, particularly bread, where its rich red colonies have been mistaken for blood.

Pseudomonas. The bacilli classified here are usually motile, Gram-negative organisms, and are characterized by the formation of water-soluble pigments of various colors which diffuse into the medium. Many saprophytic species are common in soil and water; some forms cause disease in plants.

Pseudomonas aeruginosa is important in medicine, causing infections of wounds, and other disease conditions. It is commonly called the "bacillus of blue-green pus" because its bluish-green pigments color the dressings on the infected wound. The older name of this species, still often used, is *Bacillus pyocyaneous.*

Lactobacillus. The lactobacilli are not pathogenic, but nevertheless have considerable medical interest.

Lactobacillus acidophilus is a normal inhabitant of the large intestine. It is active in the fermentation of glucose and lactose with the formation of acid, to which it is resistant. It is the organism used in "acidophilus milk" which, when fed together with lactose, may suppress putrefactive bacteria in the intestine.

Lactobacillus bifidus is the species which is abundant in the stools of nursing infants.

Lactobacillus bulgaricus is the bacillus of Bulgarian (fermented) milk.

Pasteurella. The organisms of this genus are Gram-negative, nonmotile bacilli, showing bipolar staining. They are responsible for serious, epidemic diseases in man and animals.

Pasteurella pestis is the cause of *plague* in rats, ground squirrels, and man. Related species cause infections of the "hemorrhagic septicemia" type in various domestic and wild animals.

Pasteurella tularensis (also called *Bacterium tularense*) is responsible for *tularemia* (rabbit disease) in human beings and animals.

Hemophilus. This genus includes the so-called hemophilic bacilli that grow only in the presence of hemoglobin, or of certain accessory growth substances, and in general require enriched media. The organisms are pleomorphic, appearing often as very short rods and again as slender threadlike forms.

Hemophilus influenzae is the "influenza bacillus" (or Pfeiffer's bacillus) associated with influenza, but not the sole cause of that disease. It may be responsible for other infections.

Hemophilus pertussis is the Bordet-Gengou bacillus, probably the specific cause of whooping cough.

Hemophilus conjunctivitidis is the Koch-Weeks bacillus, found in cases of acute conjunctivitis or "pink-eye." It may be identical with *H. influenzae.*

Hemophilus lacunatus is the Morax-Axenfeld bacillus, found in cases of sub-acute conjunctivitis.

Hemophilus ducreyii (Ducrey's bacillus) is the cause of the venereal disease called *chancroid.*

Klebsiella. In this genus are placed the so-called "Friedländer group" of bacteria. These are nonmotile, and capsulated, forming a conspicuously sticky growth on agar. They ferment a number of carbohydrates with the formation of acid and gas; they do not liquefy gelatin.

Klebsiella pneumoniae (Friedländer's bacillus) is associated with pneumonia and other respiratory tract infections.

Klebsiella rhinoscleromatis has been described as the cause of rhinoscleroma.

Klebsiella ozaenae has been found in cases of ozaena.

Escherichia. Here are classified the *colon group* (*Bacillus coli* group) of bacilli that constantly inhabit the normal intestinal canal of man and animals. They *ferment lactose,* as well as other carbohydrates, with the formation of acid and gas. There are many types and subtypes, but all may be designated by the general species name *Escherichia coli.* It is a common practice to use separate names for the two principal varieties, however, and to call

Escherichia coli (old name *Bacillus coli communis*) the variety that does not ferment saccharose, and

Escherichia communior (old name *Bacillus coli communior*) the variety that ferments saccharose with acid and gas formation.

Proteus. Here are placed a group of bacilli related to the colon bacilli but more proteolytic, causing rapid liquefaction of gelatin. They do *not* ferment lactose but form acid and gas from saccharose. They are actively motile, developing a spreading growth over the surface of agar.

Proteus vulgaris is the principal species. It is common in putrefying organic matter, but also is definitely pathogenic, being associated with a variety of infectious processes in man. Strains of this organism called X19 and X2 are agglutinated by the serum of patients with typhus and other diseases due to *rickettsiae* and are used in the diagnosis of these diseases.

Salmonella. The bacilli of the so-called *paratyphoid-enteritidis group* are classified in this genus. These organisms are capable of causing various forms of gastro-intestinal disease in human beings, including *paratyphoid fever,* an illness closely resembling typhoid fever, and *food poisoning,* the acute intestinal upset, popularly misnamed "ptomaine poisoning," which may follow the ingestion of a certain food contaminated with these bacilli. The *Salmonella* are differentiated from the colon group by their failure to ferment lactose or saccharose, and they differ from the typhoid (*Eberthella*) and dysentery (*Shigella*) groups in fermenting dextrose and mannite *with gas formation.*

Salmonella paratyphi (commonly called the paratyphoid A bacillus) is the most common cause of paratyphoid fever.

Salmonella schottmulleri (the paratyphoid B bacillus) may cause paratyphoid fever and also other clinical forms of gastro-intestinal disease.

Salmonella enteritidis, Salmonella aertrycke, and *Salmonella suipestifer* are the principal species found associated with cases of acute bacterial food poisoning.

Salmonella morgani has been isolated in some cases of mild enteritis, particularly in children.

Eberthella. This genus now contains the single well defined species *Eberthella typhi,* the bacillus of typhoid fever.

Shigélla. This genus contains the *dysentery bacilli,* responsible for acute and chronic bacillary dysentery and other infections in man. They are distinguished from the typhoid bacilli by the fact that they are *not motile*, as well as by immunological reactions. There are several types and subtypes, but two main varieties:

Shigella dysenteriae (Shiga type), and

Shigella paradysenteriae, (within which there are many subvarieties—Flexner, Hiss, Strong, and others).

Brucella. The tiny, nonmotile, gram-negative bacilli of this genus are the cause of infectious abortion in goats, cows and hogs, and of undulant fever, or brucellosis, in human beings. There are three very closely related species:

Brucella melitensis is found in the milk of infected goats; it causes Malta fever (undulant fever) in man.

Brucella abortus causes infectious abortion in cattle; it may be carried to man through cow's milk, producing brucellosis.

Brucella suis is the cause of infectious abortion in swine; it may also produce brucellosis in man.

THE FAMILY BACILLACEAE (GRAM-POSITIVE, SPORE-BEARING BACILLI)

All the bacteria of this family are sporulating, Gram-positive bacilli. The majority are essentially saprophytic organisms of the soil, but included are the germs of such serious diseases as anthrax, tetanus, gas gangrene, and botulism. The forms that grow *aerobically* are placed in the genus *Bacillus;* those that grow *anaerobically only* in the genus *Clostridium.*

Bacillus. There are many saprophytic species of these aerobic spore-formers.

Bacillus subtilis, *Bacillus mesentericus*, and *Bacillus mycoides* are examples of nonpathogenic forms. They are usually motile, and vary in cultural characteristics and in the size and position of the spores. These organisms are common in dust and frequently contaminate Petri dish cultures.

Bacillus anthracis is the cause of anthrax in animals and in man.

Clostridium. The bacilli of this genus make up the group of anaerobic, spore-bearing bacilli which have a prominent part in causing serious infections of traumatic wounds.

Clostridium tetani is the specific cause of tetanus.

Clostridium welchii (the "gas bacillus") is the principal species responsible for gas gangrene and is sometimes found in other infections as well.

Clostridium oedematiens, and *Clostridium odematis-maligni* (called *Vibrion septique* by Pasteur) are other important species that may cause gangrenous infections of wounds.

Clostridium sporogenes and *Clostridium histolyticum* are actively proteolytic organisms, often associated with *Clos. welchii*, or other species mentioned above, in infected wounds.

Clostridium botulinum is the cause of botulism, a serious and often fatal form of food poisoning which arises when food containing the powerful *exotoxin* of this organism is eaten.

THE FAMILY SPIRILLACEAE (THE SPIRILLA)

These bacteria are all nonspore-bearing rods which are curved into a more or less definite spiral shape, and are motile by means of flagella attached to one end of the cell (polar flagella).

Vibrio. This genus contains relatively short, curved, Gram-negative rods with no more than one, two or three polar flagella.

Vibrio cholerae is the cause of Asiatic cholera.

Numerous other species or varieties have been isolated from human feces and other sources; the majority of them are harmless.

Spirillum. The organisms in this group are longer, spiral-shaped rods with a tuft, made up of several flagella, at one pole. They are generally Gram-positive. They are nonpathogenic and are common in water or putrefying infusions. One of the best known species is a red-pigment-producing form called *Spirillum rubrum.*

REVIEW QUESTIONS—CHAPTER IX

1. What is meant by the *natural habitat* of an organism?.
2. What are *saprophytic bacteria?* What is the habitat of most saprophytes?
3. What are *parasitic bacteria?* What is the nature of their habitat? What kinds of organisms are closely confined to life in particular places?
4. Explain what is meant by the *normal flora* of a region.
5. Define *autotrophic* and *heterotrophic* bacteria and give examples of each.
6. How do saprophytes and parasites differ in food requirements?
7. Name some foods used for growing bacteria in the laboratory.
8. Explain the process of reproduction in the true bacteria. Define *simple transverse fission, Schizomycetes.*

9. What is the rate of cell division of some common species under the most favorable circumstances? Illustrate the theoretical possibilities of such a rapid reproductive rate. What is the significance of this rapid rate? What factors operate to check growth and multiplication?
10. Explain how bacterial life is affected by the presence or absence of moisture.
11. How do bacteria differ from green plants in their reaction to light? What is the effect of direct sunlight on bacteria?
12. Define *minimum*, *optimum*, *maximum growth temperature*, and *thermal death point*, *thermophilic bacteria*, *psychrophilic bacteria*.
13. What is the optimum growth temperature of different groups of bacteria and why?
14. Give the approximate minimum and maximum growth temperatures for most parasitic and pathogenic bacteria.
15. Discuss the effect of low temperature.
16. Discuss the effect of high temperature. What is the temperature of pasteurization and why?
17. Define *osmosis*, *osmotic pressure*, *semipermeable membrane*. Explain influence of osmosis on the life of bacteria. What is the practical importance of osmotic pressure effects?
18. What is meant by *reaction?* Explain its effect upon bacteria.
19. Define *aerobic bacteria*, *anaerobic bacteria*. Explain the oxygen needs of different types of bacteria.
20. Explain how bacteria live under natural conditions in relationship with other microbes. What is meant by *symbiosis*, *bacterial antagonism?*
21. Give examples of symbiosis. What is the importance of symbiotic growth of microbes in relation to decomposition processes and to some human diseases?
22. Give examples of bacterial antagonism.
23. To what is due the extraordinary power of bacteria to bring about chemical changes?
24. Define *putrefaction*, *proteolysis*, *proteolytic bacteria*.
25. How do bacteria differ in capacity to decompose proteins?
26. Mention some products of protein decomposition. What are ptomaines? What relation have ptomaines to the cause of so-called "ptomaine poisoning"?
27. Define *fermentation*. How do bacteria differ in capacity to ferment particular carbohydrates? What value are these differences in the recognition of species?
28. What are the usual products of fermentation? Name three organic acids and three gases commonly produced.
29. What effect has acid-formation by active fermenters upon other types of bacteria, especially proteolytic organisms? What are *aciduric* bacteria?
30. In general, how are fats affected by bacteria?
31. Define *chromogenic bacteria*. Give examples of common chromogenic organisms.

32. Define *toxin, exotoxin, endotoxin.*
33. Describe the principal properties of exotoxins. How can exotoxins be separated from the organisms that produced them? Give examples of disease germs that produce powerful exotoxins.
34. Describe the properties of endotoxins. Can antitoxins be prepared against endotoxins? What proportion of disease germs contain endotoxin? Name some common pathogenic endotoxin-producing bacteria.
35. The lists in the text mention four families, twenty-two genera and more than fifty species of the true bacteria. How many can you recall?

CHAPTER X

THE ACTIVITIES OF NONPATHOGENIC SAPROPHYTIC BACTERIA

ACCORDING TO the popular notion, all bacteria are harmful and only await an opportunity to injure us. But, as we have already learned, this is a totally false idea. On the contrary, of all the great invisible host of microbes, *only a small number* are ever pathogenic. The great majority of bacteria and other microbes belong to the class of harmless saprophytes. Many of them are not only harmless, but very useful in human affairs, and some play an essential rôle in Nature's scheme and really make possible the life of higher plants and animals.

Decomposition of lifeless organic matter. First in importance of the useful activities of saprophytic bacteria is the decomposition of the bodies of dead animals and plants, and nonliving organic matter of all sorts. The final result of this activity of the "rotting" bacteria is the conversion of the entire complex organic material into simple *inorganic* substances.

The process of decomposition itself is not very pleasant to contemplate. A piece of putrefying meat or a rotten egg, with its disagreeable appearance and foul odor, is a disgusting object. But it takes little imagination to realize the vital importance of these rotting processes. Suppose the dead bodies of plants and animals, and fecal matter and other waste materials excreted from man and animals, were to accumulate where they fell upon the soil, and not decompose! There would soon be no room on the earth's surface for living things. In bringing about the decomposition of lifeless organic remains, bacteria make the earth a fit place for the living.

Influence of bacteria on the fertility of the soil. The decomposition of nonliving materials by bacteria not only rids the earth of useless waste, but accomplishes a still more important result. It releases from dead or lifeless matter the elements needed for the growth of plants and returns these essential elements to the soil. The activities of saprophytic bacteria furnish the link between the dead and the living. The all important element, *nitrogen,* locked up in the bodies of dead plants and animals in the form of organic compounds, is released from its complex combinations by the action of putrefactive bacteria and passes into the soil in the form of *nitrates*—simple inorganic compounds —*and plants use the nitrates for food.* Thus nitrogen, which is really the master element of living substance, passes through a perfect cycle (Fig. 35), from the soil into the growing plant, then to the animal body when the plant is eaten, and back again to the soil through the agency of the bacteria when the plant or animal dies. Other chemical elements necessary for living things, such as carbon, phosphorous and sulphur, pass through a similar cycle in which bacteria play an important part. The supply of these chemical elements on the earth is limited, and soon would be exhausted were it not for this necessary work of the bacteria. The fertility of the soil is, therefore, largely a result of bacterial activity.

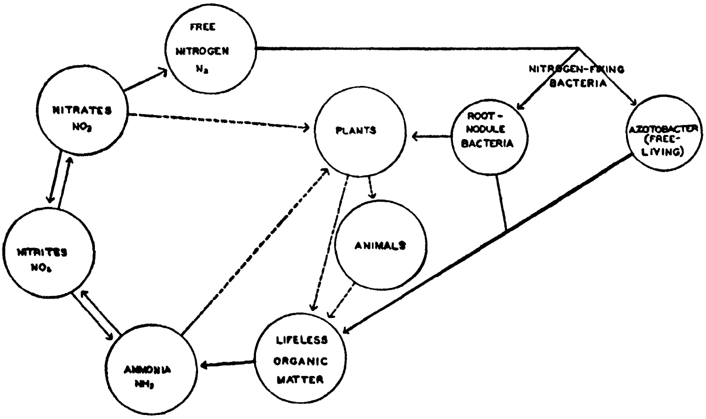


FIG. 35. The nitrogen cycle. The changes indicated by arrows drawn with a *solid* line are brought about entirely or chiefly through the activities of bacteria.

Certain kinds of bacteria have an especially marked influence upon soil fertility. These are described in the following paragraphs.

Denitrifying bacteria. Since plants are nourished by nitrates, the amount of this form of nitrogen in the soil largely determines its fertility. There are some kinds of organisms, called *denitrifying bacteria,* which can reduce *nitrates* (for instance $NaNO_3$) to *nitrites* ($NaNO_2$), or to ammonia (NH_3), or even to free gaseous nitrogen (N_2). These elementary nitrogen compounds are useless to growing plants. Therefore, the denitrifying bacteria really cause a waste of nitrogen, the all-important element for life.

Nitrifying bacteria. However, the action of denitrifying bacteria in destroying nitrates is balanced by the activities of another group of bacteria abundant in the soil, which can build up nitrates from ammonia and nitrites. These are called *nitrifying bacteria.* In contrast to the action of most bacteria, they do not cause decomposition, but instead they synthesize nitrates from simpler compounds. In this way they directly enrich the soil.

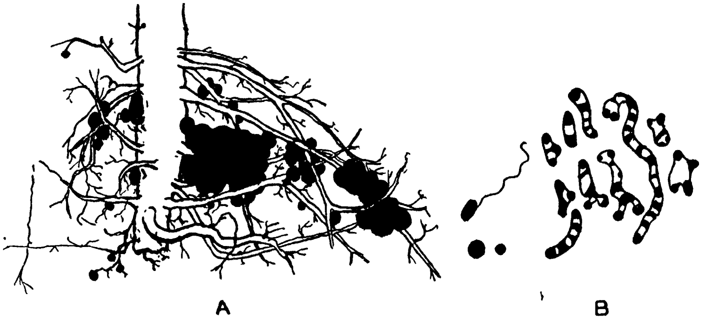


FIG. 36. A, nodules on the roots of a soy bean plant. B, forms of the bacteria that live in the root nodules. These organisms are capable of "fixing," i.e., combining with, nitrogen from the air. As a result of their activities the soil in which plants with these root nodules grow is made more fertile. (B, adapted from Topley and Wilson.)

Nitrogen-fixing bacteria. The most important contribution to soil fertility is made by still another kind of bacteria—the *nitrogen-fixing bacteria.* These organisms are able to *fix nitrogen from the air, i.e.,* they can capture atmospheric nitrogen and cause it to combine with other chemical elements in the soil, so that it becomes directly available for the nourishment of plants. This remarkable property is possessed by two groups of soil bacteria.

The members of one group live free in the soil. An example is the organism called *Azotobacter chroococcum*. The other kinds develop nodules on the roots of certain plants and are called *root-nodule bacteria* (Fig. 36). The plants which bear the nodules on their roots are called *legumes*. They include clover, peas, beans, and alfalfa. The organisms in the root nodules live in a kind of symbiosis with the plant, using carbohydrates from the plant and at the same time contributing to it nitrogen needed for its growth. The end result is to add large amounts of nitrogen to the soil.

It has been known for centuries that plants of this kind enrich the soil. It has long been a practice of farmers to carry out a rotation of crops on a particular field, and to sow the field in clover or soy-beans, or some other leguminous plant ever so often. When the clover is plowed under and the field is planted again, the land is found to be more fertile. In order to be sure that an active development of the root-nodule, nitrogen-fixing bacteria will occur it is a common practice to *inoculate* the soil or the seeds with these organisms before planting. The name *Bacillus radicicola* or *Rhizobium leguminosarum* is applied to these bacteria.

The nitrifying and nitrogen-fixing organisms are all members of the family of true bacteria called the *Nitrobacteriaceae*.

Bacteria in industrial processes. Practical use is made of the capacity of bacteria to bring about putrefaction and fermentation in connection with numerous industrial processes.

Vinegar is a solution containing acetic acid, and other substances which give it the characteristic color, aroma, and flavor. It is formed when wine (containing alcohol) is soured—*i.e.*, *fermented*—by bacteria which oxidize the alcohol to acetic acid. It will be remembered that wine itself is a product of alcoholic fermentation of a sugary solution (such as grape juice), brought about by yeasts. The familiar "mother of vinegar" is a mass of vinegar bacteria.

Various chemicals of great importance in industry may be prepared by the action of micro-organisms. Examples are *lactic acid* (formed when bacteria ferment lactose, the sugar of milk), *glycerin* (a product of the fermentation of molasses and similar substances), and *acetone* (derived from the fermentation of corn mash).

Bacteria play a very important part in the *manufacture of* *leather*. They produce necessary changes in the raw animal hides before the hides are subjected to the process of tanning, by which they are converted into leather.

The long tough cellulose fibers in the stems of flax plants have been used for centuries in the making of linen. Similarly, the strong fibers in hemp are used for making rope. When these plants are harvested the fibers are held fast to the woody part of the stems by a kind of resin. It is necessary to soften this binding so as to release the fibers. The process of freeing the fibers is called "retting." It is really a "rotting," for it is the fermentation, and consequent decomposition of the binding substances by the growth of bacteria that loosens the fibers. The *retting of flax and hemp* is, therefore, a bacteriological process of fermentation. In order to permit this fermentation to occur the flax straw is sometimes spread on the ground in the fall and allowed to remain over the winter, or, more commonly, the flax is submerged in river water for several days.

RELATION OF BACTERIA TO THE PREPARATION AND PRESERVATION OF FOOD

The substances we commonly call foods are organic materials, originating from either plants or animals. These substances—meats, vegetables, fruits, eggs, milk, and the rest—readily acquire a large bacterial population from the soil, from their surroundings, or from human hands, long before they reach our tables. Many foods furnish excellent nourishment for the growth of bacteria, and if the food be warm and moist the organisms may multiply with great rapidity. In so doing, they produce in the food the characteristic changes associated with the putrefaction and fermentation of organic matter. Sometimes these changes are desirable from our point of view; sometimes they are not. The agreeable quality and flavor of a number of our common foods are due to the previous growth of bacteria in them. On the other hand, when foods of any kind "spoil," bacteria are again responsible, in this case causing undesirable decomposition of the food.

Foods prepared by bacteria. *Fermented milks.* The most familiar kinds of foods prepared for us by micro-organisms are those which result from the growth of bacteria in milk and cream.

Soured (or fermented) milk is used the world over and in many circumstances is preferred to sweet milk. Milk sours when the bacteria in it have multiplied and have fermented the milk sugar —lactose—changing it to lactic acid. In the presence of this acid the protein part of the milk, called the casein, becomes coagulated and separates out as an insoluble mass forming the characteristic curd.

Sour milk may contain as many as 500,000,000 living bacteria in every cubic centimeter. It is still a wholesome and nutritious food, however, provided no disease germs are present in it. When milk sours in the usual way, the only types of organisms remaining in the fully curdled milk are harmless aciduric bacteria. Soured milk keeps well, because putrefactive bacteria which might otherwise spoil it are prevented from growing in the presence of so much acid.

Buttermilk. The soured milk left in the butter churn after butter has been made, and many other forms of fermented milk, are very widely used throughout the world, especially in warmer climes where it is difficult to prevent spontaneous souring. The Armenians, Turks, Bulgars, and other peoples of the semiarid lands of southern Europe, learned long ago that in such warm countries milk is kept best when it is soured. They learned that milk could be soured more quickly if a bit of the curd from soured milk were added to new lots of freshly drawn milk. They deliberately inoculated fresh milk with carefully preserved pieces of clotted casein. The character of the fermented milk thus made by various peoples depended upon the kind of bacteria introduced. Thus, the fermented milk used for generations by the Bulgars was fermented by a particular bacterium, called *Lactobacillus bulgaricus.* The term *Bulgarian milk,* in a strict sense, refers only to milk fermented by this organism, but is popularly used to mean any type of fermented milk.

In America, fermented milks are sometimes used for the feeding of infants. Many infants find acid milk readily digestible, and the continuous feeding of such milk saturates the intestine with the harmless aciduric bacteria, and greatly restrains the development of putrefactive organisms which might form poisonous products. Milk soured by inoculating it with *Lactobacillus acidophilus,* called "acidophilus milk," is sometimes given to adults who suffer from excessive intestinal putrefaction.

Butter. Butter-making is essentially a bacteriological process. It depends upon a "ripening" or fermentation of cream followed by a mechanical churning of the soured cream. The first process, the bacteriological fermentation of the cream, is the important one, and to it is due the taste and aroma of the butter. The churning is purely a mechanical process by which the tiny globules of fat are made to coalesce into a solid yellow mass of butter.

When cream is separated from whole milk, it carries with it most of the microbes in the milk so that cream always contains very many bacteria. The farmer, making small batches of butter, depends upon these organisms naturally present to ferment the cream and he permits the ripening to go on spontaneously to a point which he recognizes by experience as the proper stage for churning. He does not always get a batch of good flavor, because he does not always have the right combination of organisms in his cream.

In large creameries, a constant quality and flavor of the butter is assured by first heating (pasteurizing) the cream to kill the bacteria naturally present, and then inoculating the cream with a little of a previous batch (called a starter) or with a pure culture of a particular species of the lactic acid bacteria. When the cream has fermented and is ready for churning, it may contain as many as a billion bacteria in every cubic centimeter; but after churning, the number of living bacteria rapidly decreases. Butter which has been kept in the cold for a few days usually contains not more than 50,000 living organisms per gram.

Cheese. All of the many different varieties of cheese are products of the growth of micro-organisms. In general the method of cheese-making is as follows. The curd from soured milk is salted, then allowed to ripen. The ripening is brought about by the growth of bacteria or molds in the curd. The characteristic flavor and aroma of each of the different kinds of cheese is due to the character of the changes in the protein caused by the particular microbes concerned in the ripening. We have already mentioned that Roquefort and Camembert cheeses are formed by molds. Limburger cheese is an example of the kind of cheese in which the putrefaction of the curd by bacteria (which occurs to some degree in all kinds of cheese) is permitted to go to an advanced stage.

Sauerkraut. This is another example of a food prepared by fermentation. Cabbage is cut into fine pieces, thin layers of this chopped cabbage, sprinkled with salt, are packed tightly into casks. The salt draws much of the juice from the cabbage and this juice, rich in sugar, is fermented by the lactic acid bacteria always present. When the process of fermentation is complete, the cabbage has been converted into what we call sauerkraut. In this form it does not readily spoil, unless molds are permitted to get into it from the air.

Food spoilage and food preservation. Spoilage of food is due to the growth in it of the putrefactive type of bacteria. If food is to be preserved, it must be kept under conditions in which bacteria cannot multiply, or it must be made free of living organisms and then it must be sealed away in such a manner that no microbes can gain access to it. In general, the more microbes in a food, the more quickly it will spoil. Therefore, *cleanliness* in the preparation and handling of any food will have a marked influence upon the length of time the food will keep.

There are five common ways of preserving food. These are: (1) *drying* the food, (2) *placing it in a preservative liquid*, such as vinegar, strong salt solution, or sugar solution, (3) *smoking* it, (4) *freezing or storing it in a cold place*, and (5) *canning* it.

Drying. Moisture is necessary for bacterial growth, therefore, food may be preserved if thoroughly dehydrated. Many kinds of dried fruits and vegetables, as well as dehydrated meat and milk, are on the market.

Salting and pickling. One of the most widely used methods of preserving meats is to rub them thoroughly with salt. It is a common practice to "pickle" meats in a very strong brine solution. Fruits are commonly preserved in the form of jellies and jams which have a high concentration of sugar. The success of these methods depends upon the fact that bacteria will not develop in concentrated solutions, because in such solutions they are robbed of water through osmosis.

Smoking. Many farms have a "smoke house" where meats are hung to be preserved by smoking, and the method is also used commercially. A slow fire, usually made of green hickory wood, is maintained to furnish a dense smoke. The smoke deposits some chemical substances upon the food, which serve to preserve it for some time.

Freezing and cold storage. If a food is kept continuously at a sufficiently low temperature, it will not spoil because the ordinary bacteria do not multiply in the cold. Fish and poultry are commonly preserved by actual freezing. Eggs, fruit, and vegetables are kept at a temperature a few degrees above freezing, 4°–10° C. (40°–50° F.). Refrigeration is particularly important as a means of keeping *milk* sweet. In order to keep down the number of bacteria and to prevent early souring, milk must be kept continuously cold.

Canning. Every thrifty housewife is familiar with home canning methods. By the most common method the food is first heated until thoroughly cooked, then placed while still hot in sterilized containers and sealed. By the so-called "cold pack" method the food is packed into the clean jar while cold, and then the jar and contents are steamed the necessary length of time. In either case, the purpose of the process is to destroy all the bacteria in the food by the heating and to keep it sealed away in a container likewise freed from living bacteria.

Commercially, all sorts and varieties of foods are preserved in the familiar tin cans. After the cans are filled and sealed, they are placed in special steam chambers and subjected to a high temperature. They are then rapidly cooled and are ready for sale. Very rarely, foods spoil within the can. This means that the bacteria in such a can were not all killed in the heating process and they have slowly multiplied. If a can shows a bulging end or the contents look unusually mushy or otherwise appear abnormal, or have a bad odor, the can should be rejected without tasting the food. A safe rule would be to bring to a boil all canned foods before tasting them. Boiling will destroy not only harmful bacteria which might be present, but also the poisons they may have formed.

REVIEW QUESTIONS—CHAPTER X

1. In general, what proportion of bacteria are pathogenic?
2. What causes putrefaction and decay of lifeless organic matter? What is the final result of rotting processes?
3. Explain the importance of the decomposition of useless organic remains by bacteria.
4. Explain how the decomposition of organic waste by bacteria: (1) contributes to the fertility of the soil, and (2) prevents exhaustion of the

supply of necessary chemical elements for living plants and animals.

5. Explain the "nitrogen-cycle" and its importance.
6. Define *denitrifying bacteria, nitrifying bacteria, and nitrogen-fixing bacteria.* Name a species of free-living nitrogen-fixing bacteria, and the root-nodule bacteria. To what family of bacteria do the nitrifying and nitrogen-fixing organisms belong? Explain the effect of denitrifying and nitrifying bacteria on soil fertility.
7. What is meant by "fixation" of atmospheric nitrogen? Root-nodule bacteria? What is a leguminous plant? Name some common legumes. Explain how these plants enrich the soil.
8. Explain the part bacteria play in preparation of vinegar, important chemicals and leather, and in the retting of flax and hemp.
9. Explain the process by which milk sours. How many and what kinds of bacteria are usually found in soured milk? What are *aciduric bacteria?* Why does fermented milk keep well?
10. What is buttermilk, Bulgarian milk, acidophilus milk? What determines the quality of fermented milk? Discuss the use and value of soured milks.
11. Explain the process of butter-making and the part played by bacteria.
12. Explain the rôle of bacteria in the making of cheese.
13. How is sauerkraut made?
14. Why do foods spoil? What are the two general methods of food preservation? What is the effect of cleanliness upon the keeping quality of foods?
15. Name five common ways of preserving foods.
16. Explain the principles involved, and discuss the practical use and value of drying, salting and pickling, and smoking, as food preservation methods.
17. Explain why freezing and cold storage preserves food. What should be the temperature of refrigerators?
18. Discuss the practical importance of refrigeration in transportation of perishable foods, and in the market, the hospital, and the home.
19. What are the essential steps in the preservation of food by canning? What is the purpose of the process?
20. Are canned foods generally safe? What would cause the rejection of a canned food? Give a general rule which would assure safety in the use of any canned food.

PART TWO

LABORATORY STUDY OF MICRO-ORGANISMS

CHAPTER XI

THE MICROSCOPE

MICROSCOPES may be simple or compound. A *simple microscope* has just *one* magnifying glass or *lens.* The ordinary "reading glass" is a kind of simple microscope. *Compound microscopes* have *more than one lens,* arranged in combination, and are capable of very high magnification.

Development of the modern compound microscope. Magnifying instruments with lenses of glass were not perfected until comparatively recent times. With the exception of mirrors, optical instruments apparently were not used by ancient peoples. It is very probable, however, that some magnifying devices were known in those early times, even though we have no clear record concerning them. The modern study of glass lenses and their action began in the 13th century with the work of a Franciscan monk named Roger Bacon (1214–1294). Bacon is regarded as the founder of the science of optics. He is credited by some authorities as the first to introduce the use of spectacles. Some Italian workers are also said to have made spectacles about 1300. During the centuries following Bacon's time, the manufacture of optical instruments of all kinds was almost entirely in the hands of spectacle makers, and these artisans were responsible for the earliest form of the microscope, telescope, and other important instruments.

The first good *simple* microscopes were those made by Leeuwenhoek, about 1673 (see page 8 and Fig. 1). *Compound* microscopes were invented long before Leeuwenhoek's time, and were gradually improved during the course of two centuries, until about 1880 the wonderfully powerful and accurate modern instrument was perfected. The story of this development has been outlined in Chapter III.

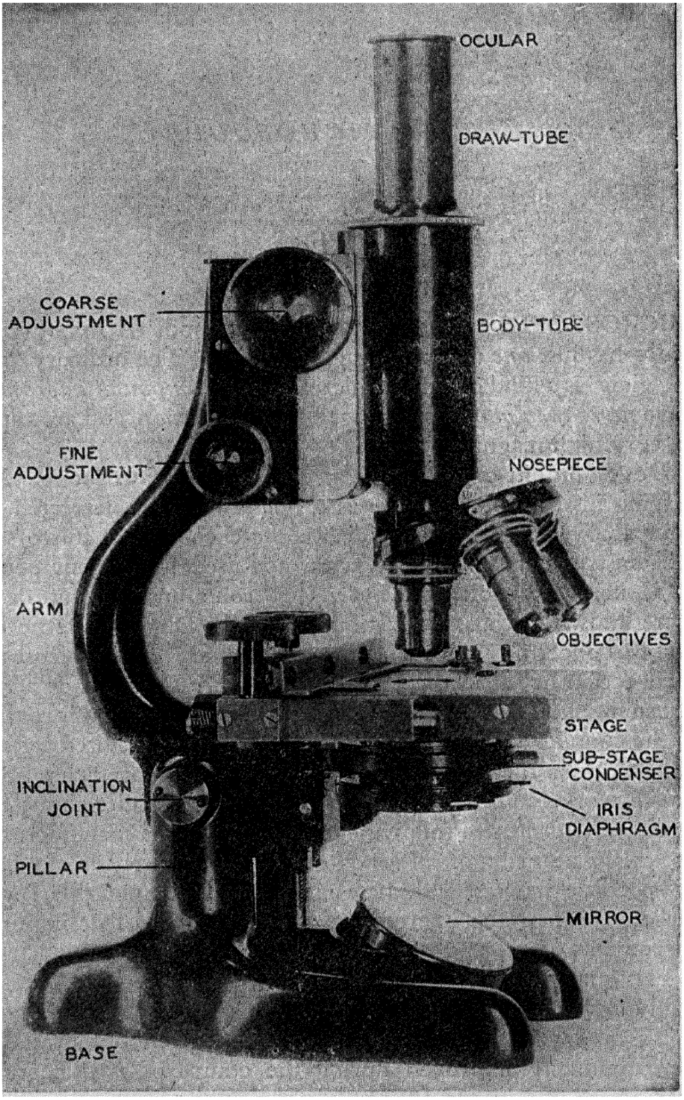


Fig. 37. A modern compound microscope.

Importance of the microscope in science. The modern microscope is truly a beautiful instrument. It is so delicate and precise in action that it serves for the highest grade of scientific investigation and at the same time it is so strongly constructed and so easy to manipulate, that the beginning student can quickly learn to use it properly and successfully. When the very great importance of the advances in knowledge made through its use is considered, it is clear that there are very few instruments so valuable as the microscope.

Bacteriology is necessarily a microscopical science. It may be said to have been born with the making of the first good simple microscope by Leeuwenhoek in the seventeenth century, and it reached its maturity as an independent science two hundred years later with the final production of the modern compound microscope.

Many other biological sciences—cytology, histology, pathology, embryology, genetics, parasitology, and branches of microbiology other than bacteriology, as well as many phases of the physical and chemical sciences—depend in large part upon the microscope. This truly wonderful instrument has added immeasurably to our insight into the *real* world, especially into the nature of living things, and the knowledge gained by its use has profoundly influenced modern life and thought.

PARTS OF THE COMPOUND MICROSCOPE AND THEIR FUNCTIONS

The *mechanical parts* of the microscope are concerned with the support and adjustment of the *optical parts*, whose function is to make the enlarged image of the object which we see. Figure 37 shows the principal parts of the microscope and how they are named. The student should have a microscope at hand while the following description is being studied.

Base, pillar, inclination joint. The *base* and the *pillar* serve to support the entire instrument. They are made heavy in order to minimize vibration as much as possible. The *inclination joint* in the pillar permits the microscope to be tipped back to any degree desired by the observer.

Arm, body-tube. The *arm* supports the *body-tube* to which the principal lenses are attached. The newer microscopes are so made that the instrument may be carried by the arm, but this may be harmful to some older makes.

Draw-tube, revolving nose-piece; tube-length. Fitting inside the upper end of the body-tube is the *draw-tube,* which may be drawn upward. Within it fits the *ocular.* The purpose of the draw-tube is to adjust the *tube-length,* that is, the distance between the top lens of the ocular above and the attachment of the *objective* into the *revolving nose-piece* below. The system of lenses in objectives and oculars is made to function best when ocular and objective are a *definite distance apart,* that is, at a definite tube-length.

In Bausch and Lomb, Spencer, and Zeiss microscopes the proper tube-length is 160 millimeters; in Leitz microscopes it is 170 millimeters. The draw-tube is usually marked with a millimeter scale. The thickness of the nose-piece. (usually 15 millimeters) must be subtracted from the scale on the draw-tube in order to adjust it to the right tube-length. That is, instead of pulling out the tube to the mark 160 (or 170) on the scale, it should be placed at the mark 145 (or 155). The proper tube-length in many microscopes is indicated by a line running completely around the draw-tube. Some microscopes have no draw-tube, and the proper tube-length is fixed when the instrument is made.

When the draw-tube is pulled out beyond the point which gives the best tube-length, the image is larger, but not quite so distinct in outline.

Coarse and fine adjustment (for focusing). The entire body-tube with its attached lenses is moved up and down by means of the rack and pinion of the *coarse adjustment.* The tube is likewise raised and lowered by *very slight degrees* by means of the *fine adjustment.* The purpose of these adjustments is to bring the object into *focus,* so that its outlines are sharp and clear. Both the coarse and fine adjustment should be manipulated carefully, and especially the latter, for it is a very delicate mechanism. The range of the fine adjustment screw is limited. At one end of its range it comes to a stop, and at the other it goes beyond the limit of movement and has no effect. *It should always be kept near the medium point.*

Stage. This is the part of the microscope on which the object to be examined is placed. In most bacteriological work the object is a transparent smear or other preparation on a glass slide.

Mirror. The *mirror* collects and reflects light up into the microscope. One side of the mirror is a plane mirror, the other is a concave mirror. Since in most bacteriological work a large amount of light is needed, the concave mirror is most useful, for it helps to concentrate the light. When a bright artificial light is used, the plane mirror may suffice.

Sub-stage condenser and diaphragm. Before the light reaches the object on the stage, it is condensed and focussed by passage through the large condensing lens, commonly called the *Abbe condenser*, in the *substage.* The result is that the maximum amount of light is directed upon the object, a necessity when the highest powers of the microscope are used. Often, however, an object is too brilliantly illuminated if all the light from the mirror passes into the condenser. For this reason there is placed beneath the condenser an *iris diaphragm.* The size of the opening in the diaphragm may be reduced to that of a pin head or any intermediate size by moving a hand lever, so that by this means the amount of light admitted to the condenser can be very accurately controlled. If a diffuse light is required, the entire substage may be lowered.

Objectives. The *objectives* are the most important of the optical parts. They limit the size of the image we see and also they are largely responsible for the quality of this image. Most microscopes are equipped with three objectives of different magnifying power—the *low power*, the *high power*, and the *oil immersion objectives.*

Low power objective. This objective is useful for the examination of protozoa and others of the larger micro-organisms, and it may be used for study of colonies of growing organisms, but individual bacteria can scarcely be made out with this lens. The low power objective is usually much shorter than the other two, and it is certain to have a *much larger lens at its end* than either of the others. Different manufacturers use various systems for marking objectives. The low power is often marked "3", or "2/3", (meaning 2/3 inch), or "16 mm."

High power objective. This objective is used in microbiology for the examination of living micro-organisms suspended in drops of water or other fluids. In most microscopes, the high power objective is longer and more slender than the low power objective, and the visible lens at its end is *smaller than that of the low power,* though still larger than that of the oil immersion objective. It may be marked "6" or "1/6" (meaning 1/6 inch), or "4 mm."

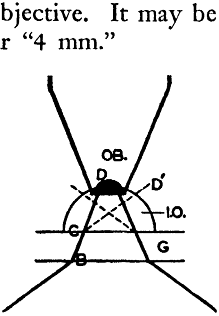


FIG. 38. Diagram illustrating the principle involved in the use of immersion oil. OB., the oil immersion objective; BCD, path of a ray of light coming up from below into the glass slide (G), and through the drop of immersion oil (I.O.) into the glass lens of the objective. Since immersion oil is optically like so much glass (i.e., has the same refractive index as glass), the ray of light passes *straight* from the glass slide into the objective. If the oil were not there this ray of light would be refracted by the air between the slide and the objective in the direction CD′, and would not enter the microscope at all.

Oil immersion objective. This objective is indispensable to the bacteriologist. *It is always used for the examination of stained smears of bacteria.* The objective may be long or short, but it will always have a *very small lens visible at its end.* It is usually marked "*oil immer.*" or "*homog. immer.*" (meaning homogeneous immersion). Also the figures "1/12" (inch), "1.9 mm." or "1.8 mm." often are engraved upon it.

The figures 16 mm., 4 mm., etc., on the objectives refer to what is called the equivalent focal length. They give an idea as to the distance there will be between the end of the objective and the object when in focus. Thus, the low power objective will be in focus about 16 mm. above the object on the microscopic slide, but the oil immersion lens will be focused less than 2 mm. above it.[1]

Principle of the oil immersion objective. The oil immersion objective differs from the low and high power objectives in that the latter are "dry." No fluid is applied to the low or high power objectives in use, and there is a layer of air between the objectives and the object when in focus. The front lens of the oil immersion objective, however, *is immersed in a special immersion oil* which fills the space between the front of the objective and the object slide. Immersion oil is a special preparation of cedar oil. It has the *same refractive index as glass*, that is, it bends rays of light passing through it to the same degree as glass. Thus, with the oil immersion objective, light coming through the glass object slide passes *straight* through the oil into the glass lens of the objective, without the deflection which a passage through air would cause (Fig. 38). This makes possible the use of lens combinations in these objectives which give a very high magnification.

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[1] Objectives are usually marked with the magnifying power (10x, 43x, or 97x) and with the letters "N. A.," followed by a figure. "N. A." means *numerical aperture.* It is an index of the power of the objective to show, distinctly separated, two points close together in the object. The higher the N. A. figure, the greater the detail the objective will reveal.

Oculars. The *ocular* or eye-piece is a short tube with two lenses, which fits into the upper end of the draw-tube. The principal function of the ocular is to act with the eye itself to *magnify the image of the object formed by the objective.* Oculars are made with different magnifying powers. By one system oculars are marked "5x," "10x," etc. indicating that these oculars magnify the image of the objective five times, ten times, etc. Other oculars are marked by the numbers 2, 4, etc. The higher the number, the greater the magnifying power. The most commonly used ocular is the 10x (or 4). This ocular used with the low power objective gives a final magnification of about 100 times, with the high power nearly 500 times, and with the oil immersion about 1000 times.

Path of light through the microscope. Figure 39 shows how the microscopic image is made. It will be seen that the rays of light cross within the body-tube, with the result that as we look into the microscope the object appears upside down.

USE AND CARE OF THE MICROSCOPE

Skill in the use of the microscope must be acquired by practice, but if a few simple rules are followed, anyone can learn to use it without difficulty and with enjoyment in a short time. If each of the following steps are carried out carefully, *in the order given*, the beginner will find most of the difficulties solved.

Steps in using a microscope:

(1) Clean the microscope.

(2) Place the microscope toward a source of light and take a comfortable position at the instrument.

(3) Place the object on the stage, swing into place the objective you wish to use, and while watching from the side, lower it to a point just under the position it will have when in focus.

(4) Secure the proper amount of light by manipulation of the mirror, substage condenser and diaphragm.

(5) Focus, first with the coarse, then with the fine adjustment; always focus up.

(6) Maintain the focus by continual manipulation of the fine adjustment.

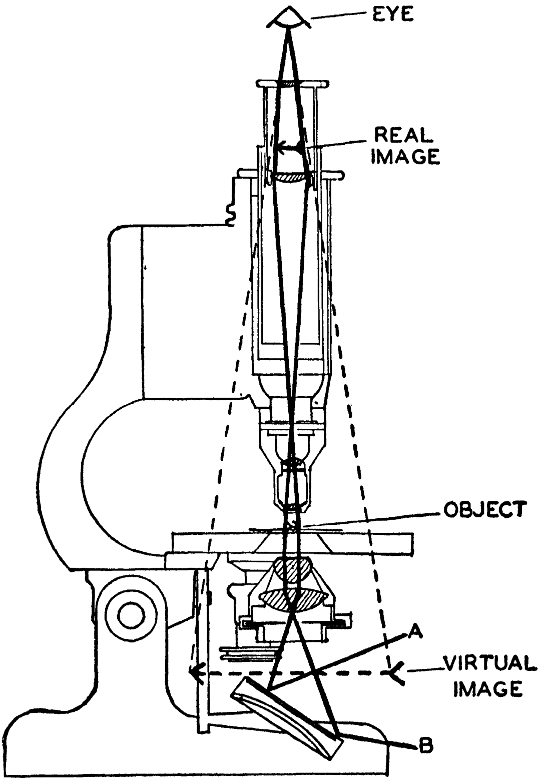

FIG. 39. Diagram showing the path of light rays through the microscope. The heavy black lines A and B, represent rays of light reflected from the mirror into microscope. After these rays pass the object they cross each other so that the image they form within the ocular (*real image*) is inverted. What the eye perceives is a *virtual image,* much enlarged, of this inverted real image.

Detailed directions:

(1) *Cleaning.* Obviously, dirty lenses will not give good images. The mirror, ocular, objectives, and condenser should be wiped free of dirt with a piece of clean lens paper or soft cotton. Do not grind the dirt into the lenses, but wipe off the dust lightly, then polish afterward. Do not attempt to take the ocular or objectives apart; clean the outside lenses only. Never touch the fingers to any of the lenses, as this will soil them.

The high power and the oil immersion objectives must be perfectly clean. Immediately after use of the oil immersion objective, the excess oil must be wiped off with clean lens paper or cotton. If oil has dried on the lens, it may be removed by wiping with lens paper or cotton moistened with a little xylol.

(2) *Position at the microscope.* Place the mircoscope on a table of convenient height, towards a source of light, and *sit at ease* before the instrument. With most preparations, the microscope may be tilted back slightly at the inclination joint, so that it is possible to look down the tube without straining the neck. Of course, when wet preparations are being examined, it will be necessary to have the microscope erect.

Throughout microscopic examinations, *keep both eyes open.* Look down the tube with either eye. Keep the eye very close to the ocular. Never strain to see; remember that *the microscope is meant to be adjusted to suit your eye.* Try to keep your eyes at rest, just as if you were looking at some distant pleasant landscape. If you do not see the object clearly, adjust the light by manipulation of the mirror and diaphragm, or correct the focus, until you do see well. The more at ease you are, the more you will see, and the less fatigued you will be.

(3) *Placing objective in position.* Revolve the nose-piece until the objective to be used snaps into place. All dry, stained smears should be examined by the oil immersion objective and no other. Wet preparations are examined by the high power objective. When the oil immersion lens is to be used, *place a drop of immersion oil directly upon the smear.* Use plenty of fresh oil. Never use oil with the other objectives.

Lower the body-tube with the coarse adjustment, *while watching from the side,* until the objective is approximately in its focal position and just below it. This means, in the case of the oil immersion lens, that the objective must be *lowered into the oil* over the smear until it almost but not quite touches the slide. The high power objective should also be lowered as close to the slide as possible without actually touching the preparation. The low power objective should be lowered to about one quarter inch above the slide.

(4) *Securing proper illumination of the object.* This is a most important step, though most often neglected by the beginner. If the illumination is correct, focusing and studying the object will be found to be a simple matter.

Before any attempt is made to focus, look into the microscope, and, by manipulation of the mirror, secure an even, bright light over the entire field. The concave side of the mirror will give the best light in most cases. The iris diaphragm should be wide open while the mirror is being adjusted, then, if the field is too brilliantly lighted, the light may be reduced by partially closing the diaphragm. *Use no more light than necessary, in order to spare the eyes.* When uncolored microscopic objects in fluids, such as living bacteria, are to be examined with the low or high power objective, the light must be reduced to dullness.

(5) *Finding the object; focusing.* Having secured good illumination, with the objective in the approximate focal position, the next step is to bring it into true focus. It is necessary to hold the object slide firmly with the fingers or by means of clips. Focusing must be done always by *raising* the body-tube very *slowly* with the coarse adjustment until the object is seen. The exact focus is then found with the fine adjustment. *The body-tube should never be lowered with the eye at the ocular,* because the objective may be driven down so far as to break the slide or injure the lens. To focus *up* means, in most microscopes, to turn the coarse adjustment screws *toward* the observer. This must be done very slowly and carefully while the eye watches for a glimpse of the object. Often the object can be more quickly located if the slide is moved slightly back and forth with one hand. As soon as the object can be seen, however dimly, the fine adjustment is brought into use and the exact focus found.

(6) *Keeping the focus with the fine adjustment.* The exact focus is a very fine point. The slightest jar will throw the lenses out of position. In order to keep the object in focus, *one hand must be continually on the fine adjustment.* This adjustment

should be moved a little, first one way, then the other, so as to reveal different levels of the object. Although an object is focused for *your* eyes, it may not be for someone else's, and when another person looks into your microscope, it will be necessary for him to move the fine adjustment until he sees the object clearly. *A person accustomed to the use of the microscope always keeps one hand engaged in delicate manipulation of the fine adjustment.*

THE DARK-FIELD MICROSCOPE

Principle of dark-field illumination. In a dark cellar the dust in the air is practically invisible, but if a streak of sunlight crosses the room, the dust immediately becomes obvious as bright particles in the beam of light. These dust particles become visible because they refract the strong light, sending some rays in the direction of the observer. By this indirect method of illumination very small objects, invisible by ordinary light, are made visible.

The dark-field microscope. This illustrates the principle of the dark-field method of microscopic examination. In the dark-field microscope a special condenser fits into the substage in place of the ordinary condenser. The center of the top lens of this special condenser is opaque, so that none of the central rays of light can pass through it, and the object is illuminated only with very oblique rays. None of the light goes directly up the objective, as in the ordinary way, but instead the light rays pass through the object almost at right angles to the objective and nearly parallel to the stage. Through the microscope the field appears dark, but any micro-organisms or other objects in the preparation stand out sharply as very bright refractile bodies, just as the dust particles appear in the beam of light across the cellar.

Use of the dark-field microscope. The dark-field microscope is used for the examination of unstained micro-organisms or other objects suspended in fluids. It is especially useful for the study of very small and delicate organisms, such as spirochetes, which are invisible or nearly invisible when viewed in the ordinary way. Extremely tiny particles, much smaller than the ordinary bacteria, may be seen in a dark-field preparation.

CHAPTER XII

MICROSCOPIC METHODS

EXAMINATION OF LIVING MICRO-ORGANISMS

THE SIMPLEST way to examine living bacteria or other micro-organisms is to suspend them in water or other fluid, place a drop of the suspension on an ordinary glass slide and cover it with a cover-glass. A much better method for most purposes, however, is to make a so-called *hanging-drop preparation.* In either case, the drop is best studied by use of the *high power objective.*

Hanging-drop preparations. A hanging-drop is prepared as follows:

(1) Secure a perfectly clean cover-glass, free from grease. (Cover-glasses may be cleaned by rubbing them carefully between the fingers with soap and water, and by rinsing them in hot water. They may be dipped in alcohol and wiped dry with a clean, lint-free cloth, or they may be polished with Bon Ami. Gentle heating of the cover-glass in the flame will remove the last trace of grease.)

(2) Place a thin ring of vaseline about the edge of the concavity on a hollow ground slide. (If a slide of this kind is not available, an ordinary slide to which a glass or rubber ring about 3 mm. high has been fastened may be used.)

(3) Place a drop of physiological salt solution or water in the center of the clean cover-glass, and emulsify the material to be examined in this fluid.

(4) Invert the hollow ground slide so that the vaseline-ringed concavity is directly above the drop on the cover-glass. Press the slide down lightly so as to seal the cover-glass to the slide. Reinvert with a quick movement. The drop containing the material hangs suspended from the cover-glass in the depression of the hollow ground slide (Fig. 40).

A hanging-drop preparation may be observed for a considerable period of time, so long as the fluid does not dry out. Motility of microbes, their natural groupings, their reactions in the presence of certain chemical substances, and many other important facts, may be learned from the study of hanging-drops.

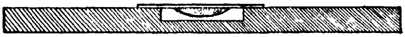

FIG. 40. A hanging drop preparation viewed from the side, and showing the drop hanging from the cover-slip in the depression of the hollow-ground slide.

It should be remembered that all small particles, including both motile and nonmotile bacteria, show *Brownian movement* in hanging-drops.

It is usually easier to focus upon the *edge* of a hanging-drop rather than upon the center. Before attempting to focus, the light should be carefully adjusted. A well and evenly lighted field should be obtained by manipulation of the mirror, then the amount of light should be cut down to dullness by closing the iris diaphragm almost completely. The *high power objective* should be lowered until it nearly touches the cover-glass (be sure that the edge of the drop is actually under the lens), and then the objective should be raised *very slowly*, while the observer looks into the instrument. As soon as the drop can be located, then the fine adjustment must be brought into use to bring the suspended material into focus.

EXAMINATION OF BACTERIA IN STAINED SMEARS

The shape and structure of bacteria is revealed to us most clearly when the organisms are dried upon a glass slide and colored with a stain, or viewed against a stained background.

Relief-staining methods. By these procedures the bacteria themselves are not colored but they stand out as in relief from a colored background. This technique is useful for the examination of ordinary bacteria, but especially valuable for study of spirochetes, or other microbes that do not stain well, and for demonstration of special structures, such as capsules.

India ink method. Place a small drop of diluted India ink near the end of a very clean slide. Emulsify the material to be examined in this ink drop. Bring the short edge of another slide down upon the drop at an angle, then draw this tilted slide edge firmly and rapidly across the first slide, so as to produce a thin, even film (Fig. 41). Let the film dry in the air, without heat. Examine the dry smear directly with the oil immersion lens. Spirochetes and other organisms are not colored by the ink and in this kind of smear they stand out as white objects in a black field. It is well to make a similar smear with the India ink alone, because there may be bacteria in the ink itself. The white marks made in the smear by particles of dust, scratches in the slide, etc., must be carefully distinguished from the micro-organisms present.

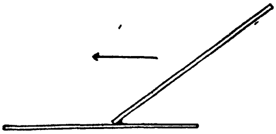

FIG. 41. Diagram illustrating the method of making blood smears and India ink smears.

Benian's relief stain. Prepare the film in the same manner as above, but use a 2 per cent aqueous solution of Congo Red instead of India ink. Allow smear to dry in air. Then wash smear in 1 per cent HCl solution, drain off at once, let smear dry. In this preparation micro-organisms appear as colorless objects in a blue field.

Preparation of smears when the bacteria are to be stained. The most usual method of examining bacteria is to make first a thin film of the organisms on a clean slide, then apply stains to the dried smear. Smears are prepared in the following manner:

(1) *Secure a clean, grease-free slide.* Slides may be cleaned in hot water, covered with Bon Ami, and polished with a clean, lint-free cloth. They may be passed quickly through a Bunsen flame just before use, in order to remove the last trace of grease.

(2) When a *smear from a bacterial culture* is to be made, place a *very small* drop of water on the clean slide. Freshly distilled water should be used, unless the tap water is known to be free from micro-organisms. Remove the organisms from the culture with an inoculating needle, with aseptic precautions, according to the technique for handling cultures described in a later chapter. Emulsify the organisms thoroughly in the water drop, then spread the drop to make a thin smear. To make a *smear from pus or other material* on cotton swabs, *roll* the swab along a clean slide to make a thin film. Usually no water is needed for a smear of this kind. Remember that smears must be *thin* enough to permit plenty of light to pass *through* them.

(3) Let the smear dry in the air. If the proper amount of water was used, the smear will dry almost as soon as it is made.

(4) After the smear is thoroughly dry, pass the slide, smear up, three times through the Bunsen flame. This process of heating is called *fixing* the smear. The bacteria in a fixed smear are dead, and are affixed to the slide so that they will not come off in subsequent manipulations. After fixing, the smear is ready for staining. (Smears are sometimes fixed with methyl alcohol or other chemicals, instead of heat.)

Stains. The stains now used are solutions of *anilin dyes.* These are artificial dyes, made from coal-tar products. Anilin dyes were first introduced into bacteriological technique about 1880 by the early bacteriologists Koch, Weigert, and Ehrlich.

Only a few of the many kinds and colors of anilin dyes are used in bacteriology. There are three stains in most common use. These are *carbol fuchsin* (a solution of the red dye *fuchsin* in dilute carbolic acid), *methylene blue,* and *gentian violet.* Any one of these stains may be used alone, or they may be used in combination with other stains and chemicals in one of the many special staining methods. Other stains in wide use are *safranin* (a faint red), *Bismarck brown,* and *toluidine blue.* Safranin and Bismarck brown are used as contrast stains (or counter stains), in combination with the blue or violet stains. Toluidine blue is frequently employed as a stain for diphtheria bacilli.

Formulæ for the preparation of the common stains are given in Appendix B.

Use of simple stains. The actual process of staining with any single stain is as follows:

(1) Cover the *fixed* smear with the staining solution for a few seconds, or minutes, according to the particular stain in use, then wash off the stain with water. Plenty of the staining solution should be used and it should never be allowed to dry upon the smear. The time needed for staining can be learned only by experience with the particular staining solution in use; even the weakest stains rarely need more than three minutes application. Sometimes gentle warming of the slide over the flame is recommended.

(2) Dry the smear by pressing the slide between several layers of filter paper, or warm the slide slightly in the flame, then blow across it and drain off the water on a towel. The smear is then ready for examination.

SPECIAL STAINING METHODS

The Gram stain. In 1884, Gram described a staining method which is of great practical value in bacteriology. Many modifications of the original Gram staining method have been described, but the fundamental procedure remains as follows:

(1) Cover the fixed smear with gentian violet for about one minute.
(2) Wash off gentian violet with water.
(3) Cover smear with a solution of iodine for one minute. (This solution is made by dissolving 1 gram of iodine and 2 grams of potassium iodide in 300 c.c. of water. It is good only so long as it retains its dark reddish brown color.)
(4) Wash off iodine solution with water and absorb excess water on filter paper.
(5) Wash the smear with 95 per cent alcohol (or acetone). *Watch carefully.* When no more color is seen leaving the smear,
(6) Wash with water *immediately.* (The decolorization must be stopped at just the right point.)
(7) Cover smear with a counterstain, such as safranin, or Bismarck brown, for one to three minutes.
(8) Wash off counterstain with water and dry smear.

Results and interpretation of the Gram stain. The Gram staining method serves to divide bacteria into two classes: (1) so-called *Gram-positive* organisms which retain the purple coloring of the gentian violet, and (2) so-called *Gram-negative* organisms, which lose the gentian violet when the smear is washed with alcohol or acetone, and which, therefore, are colored by the counterstain. When this staining process is successfully carried through, Gram-positive organisms have a purple stain, and Gram-negative organisms have the color of the counterstain (*i.e.* pink when safranin is used, or brown when Bismarck brown is the counterstain).

In the case of Gram-positive organisms the gentian violet plus the iodine forms a compound with the bacteria which is *relatively* insoluble in alcohol or acetone, hence the purple color does not come out by a few seconds exposure to these fluids (step 5), while in the case of Gram-negative organisms the iodine solution has no effect and the color comes out very readily. *But if the alcohol or acetone is allowed to act too long, Gram-*

positive organisms will also be decolorized, so that after the staining is completed they appear to be Gram-negative. On the other hand, if the decolorization is not continued long enough, Gram-negative organisms may appear to be Gram-positive. Considerable practice is necessary before Gram staining can be carried out with consistent success. When studying an unknown organism, bacteriologists check the results of Gram stains by comparison with smears of *known* Gram-positive and Gram-negative organisms stained on the same slide at the same time.

Under the same conditions, all the individuals of any one species of bacteria react in the same way to the Gram stain. Most species can be classified quite definitely as either Gram-positive or Gram-negative; a few species show a borderline reaction. Occasionally in old cultures, in which many of the cells are degenerate, or in acid media, Gram-positive organisms show a Gram-negative reaction.

The Gram stain is of great aid in the identification of unknown organisms, and much reliance is placed upon this staining method in connection with the diagnosis of gonorrhea, meningitis, and other diseases.

It is important to realize that the terms *Gram-positive* and *Gram-negative* have no significance except with reference to this staining method. Micro-organisms of the most varied kinds, both pathogenic and nonpathogenic, are found in both classes. The table on page 176 shows the reaction to the Gram stain of some of the principal pathogenic bacteria.

Acid-fast staining method. There are a number of important bacteria which are distinguished from all other kinds by the fact that they are stained with considerable difficulty, but *when once colored by a powerful stain, they resist the decolorizing action of acids.* They are, therefore, said to take an "acid-fast" stain. They do not give up the stain when the slide is immersed in alcohol, as most bacteria do, nor even when it is placed in *acid alcohol*, i.e., alcohol to which has been added about 3 per cent of concentrated acid. The following are the principal acid-fast bacteria: *Mycobacterium tuberculosis* (tuberculosis), *Mycobacterium lepræ* (leprosy), some strains of *Actinomyces* (actinomycosis), *Mycobacterium smegmatis* (a harmless inhabitant of the human skin about the genitalia), and several non-

CLASSIFICATION OF IMPORTANT PATHOGENIC BACTERIA ACCORDING TO THEIR REACTION TO THE GRAM STAIN

Gram-Positive (retain the purple stain)		Gram-Negative (lose the purple stain)	
Organism	Disease with which Associated	Organism	Disease with which Associated
Staphylococci (all pathogenic species)	—Furunculosis, etc.	Gonococcus	—Gonorrhea
Streptococci (all important pathogenic species)	—Erysipelas, tonsillitis, scarlet fever, etc., etc.	Meningococcus	—Epidemic meningitis
Pneumococci	—Lobar pneumonia, etc.	Eberth. typhi	—Typhoid fever
Cor. diphtheriae	—Diphtheria	Sal. paratyphi	—Paratyphoid fever, food poisoning
Myco. tuberculosis	—Tuberculosis	Shig. dysenteriae	—Dysentery
B. anthracis	—Anthrax	H. influenzae	—Influenza, etc.
Clos. tetani	—Tetanus	H. pertussis	—Whooping cough
Clos. welchii	—Wound infection, Gas gangrene	L. mallei	—Glanders
Clos. botulinum	—Botulism	P. pestis	—Plague
Actinomyces	—Actinomycosis	Fusiform bacilli	—Vincent's Angina, etc.
		Brucella group	—Undulant fever.
		V. cholerae	—Cholera
		Spirochetes	—Syphilis, Relapsing fever, etc., etc.

pathogenic species of bacilli found on grasses, in cow manure, butter and milk. These different varieties of acid-fast organisms vary in the degree of acid-fastness, that is, some hold the stain more firmly than others. Tuberculosis germs are very strongly acid-fast, and this is one of their most outstanding characteristics.

Several procedures may be used to demonstrate the acid-fast staining properties of an organism. The most widely used method is the Ziehl-Neelsen technique. The steps are as follows:

(1) Place the slide on a ring stand or other metal support and cover the fixed smear with strong carbol fuchsin (Ziehl-Neelsen formula). Apply the Bunsen flame beneath the slide for a few

seconds until the stain steams. *Do not boil* the stain and do not allow it to dry on the smear. Steam for three to five minutes.

(2) Wash off carbol fuchsin with water.

(3) Dip the slide into acid alcohol until the smear is decolorized. (A very faint pink color remains.) Acid alcohol is 95 per cent alcohol, containing 3 per cent of concentrated hydrochloric acid. By its action the carbol fuchsin stain is removed from everything in the smear *except acid-fast staining organisms* which retain the red color of the fuchsin.

(4) Wash off acid alcohol with water.

(5) Cover smear with methylene blue (about thirty seconds). The methylene blue serves here as a contrast or counterstain, coloring everything in the smear except the acid-fast elements.

(6) Wash off methylene blue with water. Dry smear.

Value of acid-fast staining method. This staining method is useful, of course, in the study of any of the acid-fast organisms, but it is especially valuable as an aid in the laboratory diagnosis of tuberculosis. The method is used for staining smears of sputum or other material suspected to contain the germs. When tuberculous sputum is stained in this way, the tuberculosis organisms are clearly revealed as bright red rods—the only red objects in the smear—lying in the blue field (Fig. 87).

Staining of spores, capsules and flagella. All bacterial spores appear to react in the same way toward stains. Their dense walls are not easily penetrated, and when any of the common dyes are applied for the usual length of time spores remain unstained, although the edges of free spores may be faintly colored. Spores may be stained, however, by a number of special methods. One of the best is the Abbot spore stain (Appendix B).

Capsules are not colored by ordinary staining methods. With methylene blue the capsule appears as a clear space about each organism. Uncolored spaces about bacteria not due to capsules may be confusing, however, and therefore the presence of a true capsule must be demonstrated by use of some special capsule stain. The methods of Hiss and of Gin are widely used (Fig. 28). (Appendix B.)

Ordinary stains do not color flagella at all. For this reason motile and nonmotile bacteria cannot be distinguished by their appearance in ordinary stained smears. Many special methods for the staining of flagella have been described (Appendix B). The most scrupulous care is required for successful flagella staining. Most of the staining methods depend upon the precipitation of a stain upon the entire cell, flagella included, so that the flagella appear much coarser than they really are (Fig. 31).

Special stains for diphtheria bacilli and other organisms having metachromatic granules. Various special procedures have been devised for staining diphtheria bacilli so as to bring out clearly their characteristic metachromatic granules. *Loeffler's alkaline methylene blue*, when properly aged (freshly prepared staining solution does not work well) colors the granules of diphtheria bacilli a mahogany brown and the rest of the bacilli blue. Many prefer *toluidine blue* which makes the granules stand out with striking clearness. If an iodine solution, such as that used in the Gram stain, is added after methylene blue or toluidine blue staining, the granules are better differentiated.

Two widely used special staining procedures for diphtheria bacilli are Albert's stain and Neisser's stain (Appendix B).

Staining of spirochetes. The spirochetes as a group do not color readily with the ordinary stains used for the true bacteria. Gentian violet or carbol fuchsin will stain the spirochetes of the mouth, but most of the pathogenic species, notably the *Treponema pallidum* (germ of syphilis), must be demonstrated in smears by special methods. The India ink or nigrosine relief staining methods are very useful (Appendix B). Spirochetes are best examined in the living state (unstained) by the use of a dark-field microscope.

EXAMINATION OF FUNGI

Microscopic preparations. Microscopic techniques in mycology differ from the routine bacteriological methods in one important respect—the fungi are regularly examined in wet preparations, rather than in dried, stained smears as customarily used in the study of bacteria. Only in exceptional cases is a dried smear of any value. The fungi are Gram-positive.

To make a preparation from a laboratory culture of a mold some of the growth may first be gently removed with a stiff needle, or better, a particular filament or group of filaments (with the associated reproductive elements) may be picked carefully out of the growth with a pair of small forceps, while watching through a low power microscope. This material must then be transferred to a drop of mounting fluid on a slide, covered with a coverslip and examined with reduced light, by the low and high power objectives. The fungus must be gently handled, for otherwise the spores and other structures will be displaced from their normal positions.

As a mounting fluid for fungi from cultures ordinary physiological salt solution is satisfactory, but water, alcohol, and various other fluids may be used. Especially helpful is a 5 or 10 per cent sodium hydroxide solution. This has the effect of clearing the fungus, making it more transparent. The mounting fluid may or may not have an added dye, such as dilute eosin. Yeast cells from a yeast cake are commonly suspended in saline to which dilute iodine solution is added, so as to color blue the starch granules present.

DRAWING WITH THE MICROSCOPE

How to make good drawings. It is not difficult to make good free-hand drawings of bacteria or other microscopic objects if proper habits in the use of the microscope have been acquired. Fasten the attention on only a single organism at one time, or a single small group of organisms. Draw this organism or group carefully and accurately, and then pass to another organism or group. Circles about drawings may well be omitted, for they mean nothing. Drawings should be very large and have clear, sharp outlines. Bacteria should be drawn so large and definite, that one can see whether a coccus, for example, is really round, or elongated or flattened on a side, and whether the ends of bacilli are pointed, rounded or square.

All drawings should be labelled fully, neatly and accurately.

CHAPTER XIII

CULTURE MEDIA

THE MOST important factor in securing and maintaining a growth of bacteria or fungi in artificial laboratory cultures is the character of the food substances offered them. The various mixtures of nutritive substances used for the laboratory cultivation of micro-organisms are spoken of collectively as *culture media.* The culture medium serves as the soil in which the organisms are planted for purposes of laboratory study.

Essential character of a satisfactory culture medium. In order for any material to serve as a culture medium, it must meet certain fundamental requirements.

It must contain the necessary food elements. Since the common species of bacteria and fungi utilize *complex organic foods,* a medium for general use must contain substances of this nature. Most kinds of culture media have as a basis an infusion or extract of meat to which partially broken down protein in the form of peptone is added. Some contain egg or blood-serum. In addition to these complex nitrogenous substances, carbohydrates are included in most media, and there is always an abundance of water. In order to secure a growth of some parasitic organisms, it is often necessary to add to the medium some unchanged body fluid, such as blood-serum, or ascitic fluid.

It must have the proper reaction. Most bacteria are very sensitive to acid and develop best in a medium which has a *neutral or slightly alkaline reaction.* When the ingredients of the common culture media are first dissolved, the solution is always acid. The proper reaction of the finished medium is secured by adding the necessary amount of alkali (NaOH). Most fungi, on the other hand, prefer an acid medium.

It must be sterile. A very few kinds of media, as, for example, some made from whole eggs, are so prepared as to be free from micro-organisms from the start, but the great majority of media are made with no attempt to exclude bacteria, so that the finished medium must be made sterile before it can be used, and it must be kept sterile until the organisms to be studied are planted in it. Almost all types of culture media are sterilized in the autoclave.

Uses of culture media. Culture media are prepared primarily to support growth but special kinds serve other purposes as well. Many are designed to test or demonstrate physiological properties of the growing organisms, such as the power to ferment a sugar; others are planned to differentiate one kind of bacterium from another, or to encourage the growth of a particular kind, and so to aid in the separation and identification of species. The most important of these special media are described in other chapters in connection with the studies for which they were designed.

CULTURE MEDIA COMMONLY USED

Nutrient broth and agar. The basic medium, from which many other important media are prepared, is a broth made either with an infusion or an extract of meat (Appendix B).

Plain infusion broth has the following ingredients:

Meat infusion	1,000 c.c.
Peptone	10 gms.
Common salt	5 gms.

Plain extract broth has the same composition, except that a beef extract is used in place of the meat infusion. The infusion or extract contains soluble extractives, salts, and some muscle sugar (dextrose), but little protein. The peptone supplies protein in a partially broken-down form which is readily assimilated by growing microbes. The salt is added to give the broth approximately the same salt content as that of blood, so that blood may later be added to enrich the medium, if desired, without causing hemolysis of the added red blood cells.

After the ingredients have been dissolved and the reaction has been adjusted, the mixture is filtered through cotton, and the clear, yellowish solution is distributed into test tubes and flasks. The tubes and flasks are plugged with cotton, and all are sterilized by autoclaving.

Broth is converted into a solid medium by dissolving in it 15 or 20 grams of *agar-agar* (commonly called agar) per liter. Agar is made from the stems of a seaweed which grows on the Japanese coast. It is chiefly of carbohydrate nature. It comes to the laboratory in the form of dry shreds or as a coarse powder. This substance has practically no value as food, but it has physical properties which make it ideal as the basis of a solid culture medium. It dissolves in water slowly and only after boiling for some time, but once in solution it remains liquid until the temperature is reduced to about 40° C., when it solidifies. Agar medium is easily handled in the liquid state, and with simple precautions may be poured without contamination from one sterile vessel to another. It may also be inoculated in the liquid state just before it solidifies, that is, when cooled to about 45° C. It remains solid at 37.5° C., the temperature at which most cultures are incubated.

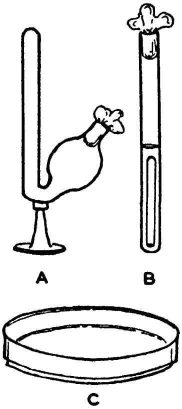

Fig. 42. Bacteriological glassware. A, B, two types of fermentation tubes. In B, gas, which may form as a result of fermentation, collects in the small tube inverted in the broth medium. C, a Petri dish.

Sterilized agar medium may be allowed to solidify in test-tubes held in a nearly horizontal position, thus forming a long slanting surface for inoculation, called an *agar slant*, or the tube may be filled ⅔ full with agar, forming an *agar deep*. Agar media are used universally for *plate cultures* in especially designed glass covered dishes called *Petri dishes* (Fig. 42, C).

Carbohydrate media. Use of indicators. *Dextrose* (glucose or grape sugar), *lactose* (milk sugar), *saccharose* (cane sugar), *maltose* (malt sugar), and many other carbohydrates, including starch, are frequently added to broth or agar media, forming dextrose broth, lactose agar, etc.

The carbohydrate is sometimes used merely to enrich the medium, but more commonly it is added to furnish a medium for testing the power of bacteria to ferment the carbohydrate. In order to detect the formation of *acid*, which is proof under properly controlled conditions that fermentation has taken place, *indicators* are usually included in carbohydrate media. Indicators are substances which change color in a gradual and definite way as the reaction changes. The most familiar indicator is *litmus*, which is deep blue in strongly alkaline solutions and pink in acid solutions. Another commonly used indicator is the *Andrade indicator.* This is a solution of acid fuchsin, a red dye, neutralized and made colorless by NaOH; it becomes red when acid is present. Other indicators frequently used in culture media are *brom-cresol-purple* (yellow when acid, purple when alkaline), and *phenol-red* (yellow when acid, red when alkaline).

The formation of *gases* in solid carbohydrate media is evinced by the presence of bubbles. To detect gas-formation in liquid sugar media, some type of *fermentation tube* is used (Fig. 42, A, B).

Gelatin medium. This medium is made by dissolving 150–200 grams of powdered gelatin in a liter of nutrient broth. It is solid at room temperature, but becomes liquid at about 30° C. and therefore *can be used in its solid form only at room temperature*, and not in the incubator. Its principal use at present is to test the capacity of organisms to "liquefy" gelatin, *i.e.*, to change it so that it will not again solidify.

Milk media. *Plain milk* and *litmus milk* (containing litmus as an indicator) are very useful media. Many bacteria, including pathogenic species, grow readily in milk, and bring about characteristic changes in the medium.

Loeffler's blood-serum medium. This medium consists of

Beef (or other animal) blood-serum	— 3 parts
Infusion broth plus 1 per cent of dextrose	— 1 part

Test tubes containing the mixed broth and serum are placed in a slanting position in a special heating apparatus, or in the autoclave, and gentle heat, gradually increased to about 70° C., is applied. During this heating the medium coagulates, forming a solid opaque mass in the form of a slanting wedge in the tubes. When thoroughly coagulated, the medium is sterilized by autoclaving in the usual way.

Loeffler's serum medium is invaluable for the cultivation of the *diphtheria bacilli* and *is relied upon the world over for cultures from the nose and throat on which is based the laboratory diagnosis of diphtheria.* No other medium is so satisfactory for this purpose. It is also useful for cultivation of many other bacteria, especially those of the parasitic types.

Coagulated egg media. There are several varieties of media commonly used which are made of eggs coagulated by heat, in the same way that Loeffler's slants are made.

Dorset's plain egg medium and *Lubenau's glycerin egg medium* are used principally for the cultivation of the germs of tuberculosis and related acid-fast bacilli. *Petroff's gentian violet egg medium* is one of several similar media designed especially for the cultivation of human tubercle bacilli from sputum. The gentian violet in this medium restrains the growth of the Gram-positive cocci always abundant in sputum and the tubercle bacilli have, therefore, a better chance to develop.

Cooked meat medium. This medium is an especially favorable one for anaerobic bacteria. Finely ground lean beef is mixed with an equal volume of water and heated until the meat particles are thoroughly cooked. The meat is placed in test tubes in a layer 2 or 3 inches deep and enough nutrient broth is added so that about 3 c.c. of clear broth lie on top of the meat. The medium is sterilized by autoclaving.

Brain broth and brain agar. Small pieces of fresh, washed calf brains are placed in the bottom of test tubes and covered with nutrient broth or semisolid agar containing 1 or 2 per cent dextrose. Sterilization is attained by autoclaving. This medium is excellent for streptococci, and for many other of the most delicate pathogenic bacteria, including the meningococci.

Blood agar and other enriched media. Agar media enriched by the addition of sterile animal or human *blood* are the most generally useful of all culture media in medical bacteriology, since they support the growth of nearly all pathogenic and parasitic bacteria. *Blood agar* is of value also as an aid in the recognition of different varieties of streptococci and other organisms through the characteristic changes they produce in the blood. Blood serum, ascitic fluid, yeast extracts, glycerin, products of digested meat, and many other complex nutritive substances are used to enrich media for special purposes.

Special media for fungi. In securing a pure culture of molds or yeasts, advantage is taken of their peculiarly high resistance to acid, and their preference for a medium containing a large amount of fermentable sugar. Good media for isolation purposes may be made from the ordinary nutrient broth or agar, as used for bacteria, by adding about 5 per cent of dextrose, and by making the medium strongly acid—so acid that bacteria are inhibited, while the relatively slow-growing fungi have a chance to develop unhindered.

A medium widely used for obtaining the first growth of fungi is a solution of peptone (1 per cent), and crude maltose or dextrose (4–5 per cent) in water, of acid reaction (pH 5.5), to which agar in the usual proportions (1.5 per cent) may be added. The original formula for this medium was developed by Sabouraud, a French mycologist who has contributed much to our knowledge of the fungi. *Sabouraud's medium* is especially useful for cultivation and identification of the fungi causing skin diseases in man. Other pathogenic fungi may require a richer medium, such as blood agar. A form of Sabouraud's medium without sugar is commonly employed for stock cultures of fungi.

CHAPTER XIV

CULTURE METHODS

A PECULIAR technique, requiring the most meticulous attention to numerous important details, is employed in every step of the preparation and examination of cultures and in all other procedures in which microbes are handled. This technique is necessary in order that dangerous germs may be studied safely, and also in order to avoid contamination of the cultures or other material being examined with the micro-organisms naturally occurring in the dust, on the fingers, and elsewhere. Microbiological technique is essentially an *aseptic technique,* that is, a procedure by which the worker excludes from his cultures microbes he does not wish to study, prevents infection of his own person, and avoids contamination of his surroundings. He creates for himself an aseptic environment in which to work. The glassware, the culture media, everything used for the study of germs in laboratory cultures is made sterile to start with. And once a culture is prepared, it is always handled in such a way that no microbes can gain entrance to it from without and so that none of the bacteria in the culture can escape.

AEROBIC CULTURE METHODS

Handling test tube cultures. When a smear is to be made from a test tube culture, or when organisms are to be transferred from one tube to another, a definite procedure for handling the culture and the inoculating needle must be carried out (Fig. 43). The following is one commonly used method for a right-handed person:

With the culture tube in the left hand, and the inoculating needle in the right hand, sterilize the needle by holding it *perpendicularly* in the flame of a Bunsen burner until it is red hot.

Remove the cotton plug from the tube with a twisting motion by grasping it between the little finger and palm of the right hand. The portion of the plug extending outward from the hand should not touch the fingers or any other object. Pass the lip of the tube through the flame, then hold it *nearly horizontal* and close to the flame. Insert the sterilized needle into the tube and allow it to cool, then remove a minute portion of the growth with the tip of the needle. If the culture is on an agar slant, touch the growth very lightly; *do not plunge the needle into the agar.* Flame the lip of the tube again and replace the plug. If a smear is to be made, emulsify the organisms in a drop of water on a slide and proceed as previously described. If a tube of new medium is to be inoculated, take up the second tube with the left hand, remove the plug, flame the lip, inoculate, flame the lip again, and replace the plug as before. Finally, sterilize the needle by holding it again in the flame before putting it down.

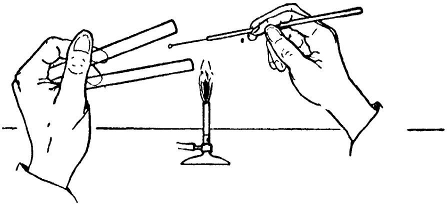

Fig. 43. Handling test tube cultures. The cotton plugs have just been removed from the tubes preparatory to making a transfer. The plugs are held by the fingers of the hand that holds the needle and cannot be seen. Note that the tubes are held nearly horizontal and close to the flame.

A quicker method of making a transfer from one tube to another is to hold both tubes parallel in the palm of the left hand. Remove both plugs, holding one between the little finger and palm of the right hand and the other between the third and fourth fingers. Flame the lips of both tubes and with the sterilized needle quickly make the transfer. Then flame the tubes again, replace the plugs, and sterilize the needle.

Inoculation of test tube cultures. Cultures are most commonly made in some kind of liquid or slanted medium. *Broth*, milk, and other liquid media are inoculated by shaking the needle or swab bearing the organisms in the liquid a few times. Transfers from liquid cultures to new media are often made with sterile glass pipettes. *Slants* of agar or other media are inoculated by drawing the needle or swab *lightly* from bottom to top across the slanting surface. The surface of the agar must not be broken.

Two other kinds of test tube cultures are often made. These are *stab cultures* and *shake cultures*. A *stab culture* is made by plunging a long straight inoculating needle into the center of a deep column of semisolid agar or gelatin in a test tube; the needle is withdrawn by the same route. Most of the growth will occur along the line of the stab (Fig. 47, D). To make a *shake culture*, a deep column of agar is thoroughly melted, then cooled to about 45° C. At this temperature it is still liquid and yet cool enough so that the organisms to be inoculated will not be killed by its heat. The inoculating needle is shaken vigorously through the melted agar, the medium is then rapidly cooled, so that the agar solidifies quickly. In these cultures growth occurs in scattered colonies throughout the medium (Fig. 47, C).

Plate cultures. Cultures in agar or other solid media in Petri dishes are called plate cultures and the Petri dish itself is commonly referred to as a *plate*. This term originated with the work of Robert Koch who was the first to use gelatin and agar media. He had no Petri dishes and he poured his media on sterilized plates of glass.

Pouring a plate. The process of pouring melted medium from a test tube into a Petri dish is spoken of as "*pouring a plate*." The cotton plug in the tube of medium is removed and the lip of the tube is heated in the flame. The cover of the sterile Petri dish is then lifted just enough to permit the neck of the tube to enter and the media poured in. Then the Petri dish cover is immediately dropped into place again. The dish may be gently revolved to distribute the medium evenly through the dish, but it must not be moved about or picked up until the medium has thoroughly hardened.

Even this simple operation requires attention to a number of details. When agar plates are to be poured, the first essential is that the agar be *completely melted*. Otherwise there will be lumps in the plate. Several minutes of heating in boiling water is necessary to melt tubes of agar, and large amounts of agar will require prolonged heating. Once entirely melted, the second necessary step is to *cool the agar uniformly to about 45° C.* With a little experience it is possible to tell when a tube of agar is about this temperature by the feel of the tube against the skin. When it is the proper temperature, it has cooled just enough so that it can be held against the cheek or wrist for a few seconds without discomfort. The plate must then be poured *immediately* before the agar has a chance to cool so far that it solidifies. Careful attention to the cooling of the agar and quick action when it has reached the proper temperature are necessary. In case the agar solidifies before it can be poured into the dish, it must again be melted completely in boiling water, and cooled once more, before another try can be made. It is necessary to cool the agar to 45° C. whenever it is to be inoculated while liquid, because a higher temperature might kill the bacteria introduced, and in any case it is desirable, so that the medium will solidify promptly and not cause an excessive formation of water vapor. As agar cools in a Petri dish, the steam given off forms water drops on the under side of the cover. In order to prevent this water from dropping onto the surface of the culture, Petri dishes are *turned upside down* as soon as the medium has hardened and are incubated in this position.

There are two methods of inoculating agar Petri dish cultures. The resulting preparations are referred to as (1) *pour plates,* and (2) *streak plates.*

Pour plates. To make this kind of culture the melted agar medium in a test tube, cooled to about 45° C., is inoculated by shaking the needle bearing the organisms through it just before it is poured into a sterile Petri dish. The growth will develop throughout the hardened medium, both on the surface and in the depths of the agar (Fig. 45).

Streak plates. The sterile, melted and cooled medium is first poured into a sterile Petri dish and allowed to harden thoroughly, then the surface of the hardened agar is inoculated by streaking the needle or swab across it (Fig. 46). Here the growth occurs on the surface only.

ANAEROBIC CULTURE METHODS

General method of inoculation for anaerobic cultures. The routine method employed for making a culture of anaerobic organisms in any liquid or semisolid medium is as follows:

(1) Heat the medium in boiling water for twenty to thirty minutes to drive off the oxygen contained in it.

(2) Cool the medium quickly under running water. Enriching substances, such as ascitic fluid or blood-serum may be added to the partially cooled medium.

(3) Inoculate the depths of the medium.

Many anaerobic bacteria grow very well, especially when there is a mixture of organisms, in semisolid media inoculated in this manner and incubated in the ordinary way. But all anaerobes develop more readily if, in addition to the steps outlined above, the air is totally excluded during incubation by use of some one of the mechanical or chemical methods described below.

Some of the most useful anaerobic culture methods are illustrated in Figure 44.

Anaerobic methods for single test tubes or flasks. *Vaseline seal method.* Any liquid or solid medium in deep columns in test tubes or flasks may be boiled to drive out the contained oxygen, cooled quickly, inoculated, then covered with a half inch layer of *sterile melted vaseline.* The vaseline may be sterilized in the autoclave, and added with a sterile pipette. The vaseline hardens as it cools to make an air-tight seal (Fig. 44: B).

Pyrogallic acid oxygen-absorption method for slant cultures. The following is a satisfactory anaerobic method for slant cultures. It depends upon the use of an alkaline solution of pyrogallic acid to absorb the oxygen from the tube (Fig. 44: C).

Test tubes (preferably the large size, so-called "Board of Health" tubes) containing the sterile, slanted medium should be plugged with a good grade of *absorbent* cotton. If the original plug is not of absorbent cotton, a roll of absorbent cotton may be made with the fingers. It need not be sterile. Cut or burn off the upper portion of the absorbent plug and push it into the tube to about ⅜ inch below the rim. The plug should be about 1½ inches long. Tap dry pyrogallic acid lightly into the space above the plug. Then add with a pipette not more than 1.5 to 2.0 c.c. of *warm* 10 per cent sodium hydroxide solution, and close the tube quickly with a solid rubber stopper. Twist the stopper into the tube as tightly as possible, then dip the top of the tube into melted paraffin. Incubate these tubes in an upright position like any other culture.

When examination of these cultures is desired, scrape off the excess paraffin and remove the stopper. Using forceps, remove the cotton plug with a slow twisting motion and close the tube with a new sterile plug. If the proper amount of hydroxide solution was used, removal of the original plug will leave the walls of the tube practically dry. After examination, if it is desired to incubate the culture further, it is merely necessary to roll another plug of absorbent cotton with the fingers, insert it, and repeat the process outlined above.

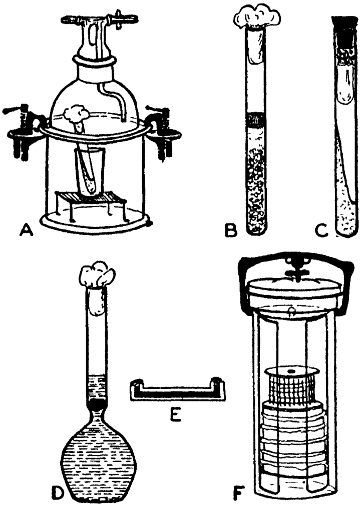

FIG. 44. Anaerobic culture methods. A, a Novy jar. The air in this jar can be exhausted by a vacuum pump, and replaced by some inert gas, such as nitrogen. B, a tube of meat medium sealed by a layer of sterile vaseline over its surface. C, a slant culture made anaerobic by the pyrogallic acid method. The absorbent cotton plug has been pushed down into the tube, and some dry crystals of pyrogallic acid placed on it. Then a warm NaOH solution has been added, and the tube quickly closed with a rubber stopper, and sealed by dipping in melted paraffin. The alkaline solution of the pyrogallic acid absorbs the oxygen in the tube. D, a flask with constricted neck and marble seal. The marble may be pushed aside for purposes of inoculation. Many anaerobes grow well in such a flask. E, the inverted Petri dish method. The inoculated agar is poured into the larger part (ordinarily the cover) of the Petri dish, then while the agar is still melted, the smaller part is placed upon it and the edges are closed with paraffin, thus sealing the agar between two layers of glass. F, the Varney phosphorus anaerobic jar. The Petri dishes are stacked upside down in the holder and separated by filter paper. A piece of phosphorus is placed in a metal cup on top of the dishes, and the jar is quickly closed. The phosphorus soon ignites and as it burns it uses up the oxygen in the jar, thus establishing anaerobic conditions.

Anaerobic methods for plates. One of the best and simplest of the many methods of cultivating anaerobes is to grow them in agar held between two layers of glass. One useful adaptation of the general method is the inverted Petri dish (Fig. 44: E).

Inverted Petri dish method. The two halves of an ordinary Petri dish are fitted one inside the other, the smaller half (bottom) inside the larger half (top), open side up. The inverted dish is wrapped in paper and sterilized. Agar medium is boiled, and cooled as usual, inoculated and then poured into the larger dish. The smaller dish is then laid upon the medium while it is still fluid, and the rim of exposed agar covered with melted paraffin. The result is an anaerobic culture, since oxygen is entirely excluded from the layer of agar held between the glass. When the growth is to be examined the upper dish is pulled off.

Anaerobic jars. Several types of jars designed to furnish anaerobic conditions for plates and other cultures by replacing the oxygen in the jar with hydrogen or other gases, or by chemical absorption of the oxygen, have been devised (Fig. 44: A, F).

CHAPTER XV

ISOLATION AND IDENTIFICATION OF BACTERIA

IN NATURE, almost all kinds of bacteria or fungi, including the disease-producing species, live naturally in more or less intimate association with other kinds. Therefore, the primary culture from almost any source will be a *mixed culture* containing organisms of several different kinds. But in the laboratory the various species may be separated from one another and cultivated by themselves. A culture which contains just one species is called a *pure culture.* An organism must be obtained and studied in pure cultures before the properties peculiar to itself may be learned.

The process of obtaining a pure culture by separating one kind of bacterium or fungus from a mixture with other kinds is spoken of as *isolation* of the organism.

Principle of isolation methods. The earliest workers to study bacteria in the laboratory used only liquid culture media. The task of securing pure cultures using liquids only was naturally very difficult, and only men of the highest mental attainment and possessed of the patience of genius, like Pasteur and Lister, could make much headway in the study of microorganisms. We have already explained how Robert Koch, in 1881, by the use of solid culture media and the plate method, simplified the entire procedure of securing pure cultures. The methods for isolating bacteria used today are essentially the same as those of Koch. These methods depend upon (1) *dilution of the original material in solid media cultures in order to secure separated, pure colonies,* and (2) *transfer of a pure colony to a fresh sterile medium,* without contamination from the neighboring growth. The process of picking out a single colony for a smear or transfer is called *fishing.*

Dilution methods with agar media. Just as in planting a vegetable garden it is necessary to scatter the seed over the ground so that the plants which later develop will not be too close together, so the bacteria which are to be planted upon culture media must be thinned out so that separate, typical, pure colonies will appear. Any method of diluting the original material will serve so long as extraneous bacteria are not introduced.

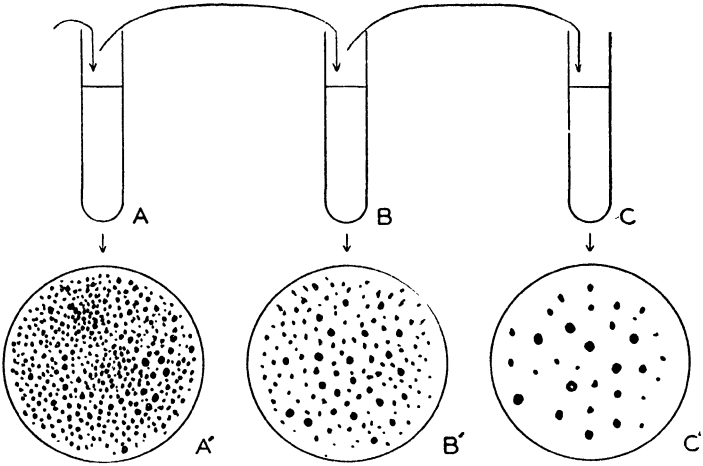

FIG. 45. Pour plate cultures, illustrating the effect of dilution of the inoculated material. A, B, C, tubes of melted agar, inoculated in series as indicated by the arrows. A′, B′, C′, pour plate cultures made from A, B, and C, respectively, showing the growth resulting after incubation. Compare with streak plate cultures (Fig. 46) as a means of securing isolated colonies of bacteria.

Dilution methods for pour plates. One commonly used method of securing pure colonies is to make a series of pour plates, inoculating each plate in the series with decreasing amounts of the original material.

Dilutions of this material may be made in a series of tubes of any sterile liquid medium, such as broth, or water, or physiological salt solution. The first tube of sterile broth, for example, may be inoculated with two loopfuls of the original material. After shaking this tube well to distribute the bacteria, two loopfuls from this tube may be transferred (with the usual precautions against contamination) to a second tube. This process may then be repeated until a series of four, five, or more tubes have been inoculated. Then 1 c.c., or a loopful, of the contents of each tube may be added to separate tubes of melted agar and the mixtures poured into sterile Petri dishes.

By another common method the dilutions are made directly in the liquid agar (Fig. 45). A series of tubes of agar are heated until the agar melts, then cooled to about 45° C. A needle bearing a loopful of the material from which the bacteria are to be isolated is shaken vigorously through the melted agar in one tube, then passed, without being sterilized, to a second melted agar tube, then to a third, and so on until four or five or more tubes have been inoculated. Then separate plates are quickly poured with the contents of each tube before the agar solidifies.

Dilution by means of streak plates or slants. Streak plates, instead of pour plates, may be used for isolation. It is merely necessary to spread out the original material over the *surface* of agar plates to such a degree that separate pure colonies will develop.

A very minute amount of the material (which may or may not have been previously diluted with broth or other sterile liquid)

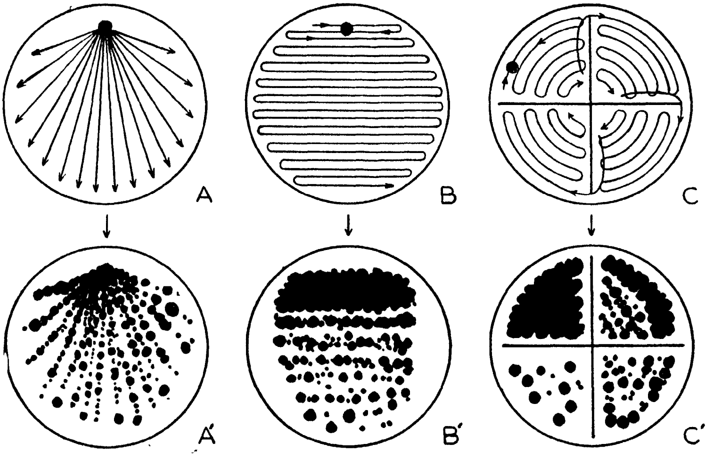

FIG. 46. Streak plate cultures. A, B, C, methods of inoculating streak plates. In method C the needle is sterilized after streaking a quarter of the plate, then recharged with the organisms by passing it across the section just previously streaked. A′, B′, C′, the growth resulting after incubation, when inoculated as in A, B, and C, respectively. Compare with pour plate cultures (Fig. 45) as a means of securing single colonies of bacteria.

may be placed on the surface of one plate. With a looped or a straight needle, or a sterile, bent, glass rod, this material may be streaked over the entire surface of the plate; then without sterilizing the needle or rod, a second plate, and a third, or more, may be streaked in the same way. Some of these plates should develop well separated pure colonies.

The making of good streak plates is not so easy as it may appear and much patience and skill is required. In order to avoid contamination, the cover of the Petri dish must not be lifted more than is necessary. The agar must be thoroughly hardened before inoculating and great care must be taken not to break the surface of the medium in streaking. The needle should be held so lightly that practically the only pressure upon the agar surface comes through the weight of the needle itself. *The streaking must be done patiently according to some definite plan.* As much as possible of the surface of the medium should be utilized. Some of the common methods of streaking are illustrated in Figure 46.

Fishing. The process of picking up bacteria from a single colony with a sterile needle, without touching neighboring colonies, is often a delicate task requiring much care and skill. This is particularly true when pathogenic organisms are being isolated because their colonies are usually small.

A straight needle is always used for fishing. Often it is an advantage to have the end of the needle bent at a right angle and filed to a fine point. Colonies to be fished from plate cultures may be marked by drawing a ring about them with a wax pencil on the bottom of the dish. The colonies may be given numbers, and smears or cultures made from them may be correspondingly numbered. In fishing, as in any other procedure, the culture must be protected from contamination, and the complete removal of the cover of a Petri dish is avoided whenever possible. Fishing from plate cultures is much easier if performed with the aid of a low power dissecting microscope.

STUDY AND IDENTIFICATION OF PURE CULTURES

Once a pure culture is obtained, a long study is usually required before the organism can be fully identified and named. It is necessary to determine: (1) its morphological properties and staining reactions, (2) its cultural characteristics, (3) its physiological requirements and chemical activities, and (4) its pathogenicity (disease-producing power). Also tests would be made (5) to see whether the organism will agglutinate, or otherwise react specifically, with an immune serum known to contain antibodies for the species to which the organism appears to belong. This will identify the unknown organism positively in most cases.

Morphological study. Staining reactions. Microscopic studies will reveal the form (coccus, bacillus, or spirillum), and the characteristic arrangement and grouping of the cells, their size, and the presence or absence of spores, capsules, granules, or other peculiarities of structure. Examination of hanging drop preparations will show whether the organisms are motile or not. If so, the flagella may be stained. The reaction to the Gram stain is of great help in determining the class to which the unknown bacterium belongs. In case the organism is acid-fast, this will be revealed by the acid-fast staining method. These studies supply a great deal of information about an organism and

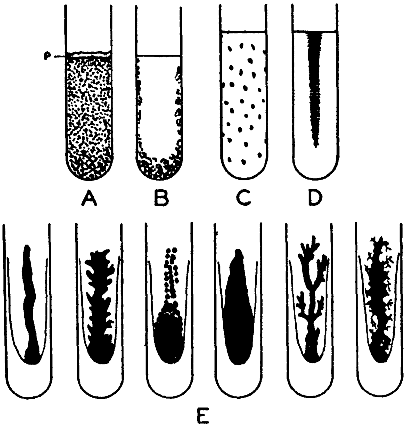

FIG. 47. Types of bacterial growth. A, B, appearances shown by cultures of bacteria in broth. Most species produce a uniform cloudiness throughout the broth, as in A; others grow only as flocculent masses along the sides and at the bottom of the tube, as in B. Some organisms form a membrane-like film over the top of the broth. This is called a pellicle, (P). C, an agar shake culture, showing colonies distributed throughout the medium. D, an agar stab culture. E, forms of growth shown by different species of bacteria on the surface of agar slants.

may give a strong hint as to its identity, but *a species of bacteria can never be identified with certainty on the basis of morphology and staining alone.* There are only three basic shapes which bacteria may take, and only a few other morphological features which help to differentiate organisms from one another, yet there are hundreds of different species. Organisms which stain alike and look alike under the microscope may be totally different in other and more important characteristics. Thus, nearly every type of disease germ is duplicated, so far as morphology and staining are concerned, by other species which are harmless.

Cultural characteristics. Further information about an organism is obtained by observing the way it grows in various kinds of culture media. Certain species have a very characteristic manner of growth in broth or milk, in agar, or other media. Figure 47 illustrates some of the forms of growth commonly seen.

The agar *colonies* are sometimes so distinctive that they give a valuable clue to the identity of the organism. Plate cultures are most conveniently used for the study of colony characteristics (Fig. 48). The colonies may be observed with a hand lens, or they may be studied under a dissection microscope or under the low power objective of the ordinary microscope. In the description of colonies, the following points are usually considered: *form*, *size*, *color*, *optical characteristics*, *surface*, *elevation*, *edge*, *internal structure*, and *consistency* when touched with a needle. Thus, a colony of staphylococcus might be described as follows: a circular, orange-colored, opaque mass, about 2mm. in diameter, with a smooth, glistening surface and regular edge, slightly convex, showing a homogeneous, granular, internal structure, and having a soft consistency.

Physiological requirements and chemical activities. The most significant properties of an organism are learned by a study of its physiology and its biochemical reactions. The following points are among those usually investigated:

(1) *Food requirements.* Does the organism grow upon the simpler agar and broth media, or does it require media enriched with blood or other body fluids?

(2) *Temperature relations.* Does it grow best at body temperature? If not, what is its optimum growth temperature? Minimum and maximum growth temperature?

(3) *Relation to oxygen.* Does it grow best under aerobic or anaerobic conditions, or under partially reduced O_2 tension?

(4) *Pigment production.* Does it develop a colored growth on agar slants or other cultures?

(5) *Proteolytic action.* Does it liquefy gelatin? Cause digestion of coagulated blood-serum, meat, and similar substances? Does it form indol? (Indol is a product of the decomposition of tryptophane, one of the amino-acids composing proteins.)

(6) *Fermentation of carbohydrates.* Does the organism produce acid, or acid and gas, in culture media containing dextrose, lactose, maltose, sacchrose, or other carbohydrates?

When these facts are known it often becomes possible to recognize the species, or at least the group of closely related species, to which the unknown organism belongs.

Pathogenicity. One other important property remains to be tested, however. Will the organism produce disease in laboratory animals? The animals most commonly used in laboratories are guinea pigs, rabbits, white mice, and white rats. For certain special work monkeys, dogs, cats, or other animals may be needed. Pathogenicity tests are usually made by inoculating the organisms under investigation into these animals *subcutaneously* (just under the skin), *intraperitoneally* (into the peritoneal cavity), or *intravenously* (into a vein). Other possible routes of injection are *intradermal* (into the skin just under the epidermis), *intramuscular* (into the muscles, as of the thigh), and *intracerebral* (directly into the brain through the skull).

Such animal inoculations are often of great value in identifying a particular organism. Also they help in securing pure cultures of disease germs. If a material contains acid-fast bacilli, for example, and there is doubt as to whether they are tuberculosis bacilli or not, the matter can be settled by inoculating a guinea pig. If the material injected contains tubercle bacilli, the guinea pigs will develop tuberculosis in a few weeks. It is difficult to cultivate the tubercle germs upon culture media directly from sputum or other matter containing them, but if this material be inoculated into a guinea pig, the germs can easily be obtained in pure culture from the lesions in the animal.

Identification by aggultination reactions and other serological tests. One final method of identifying a species of bacteria is by use of serological tests. If, for example, we have a Gram-

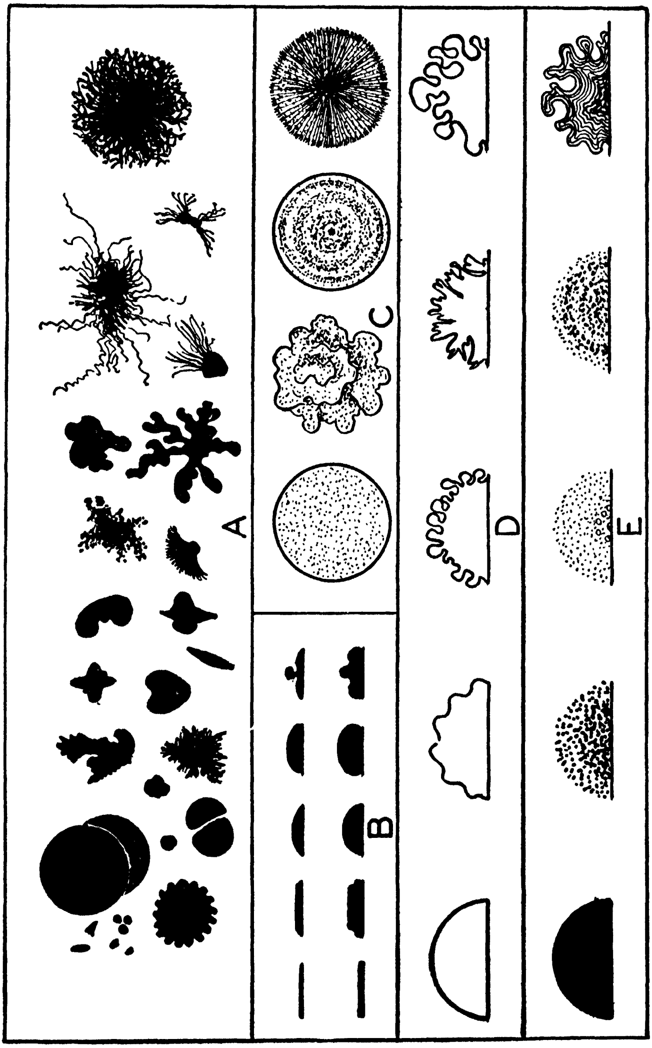

FIG. 48. Colonies of bacteria in agar cultures. A, various forms of colonies; B. cross sections; C, surface appearances; D, character of edges; E, internal structure.

negative bacillus which has the characteristics of the typhoid germ, a final test of its identity may be made by mixing a suspension of the organisms with a blood-serum known to contain antibodies for that species. If agglutination occurs under properly controlled conditions, the organism must be a typhoid bacillus.

COUNTING BACTERIA BY CULTURAL METHODS

In the examination of water, sewage, milk and other materials, it is often necessary to make a count of the number of bacteria. The method is simple, but the cultures must be made very carefully in order to assure consistent results. The *standard methods* for counting the bacteria in water and milk, published by the American Public Health Association, are used almost universally. It is necessary (1) to secure a representative sample of the material to be examined in a sterile container. Then (2) the sample is diluted in a definite quantitative manner and (3) the diluted material is inoculated into agar media. Then (4) the colonies which develop are counted, and, assuming that each colony represents a single living organism in the original sample, (5) the number of bacteria per cubic centimeter of the sample is calculated.

Technique. The method of counting the bacteria in water may be taken as an example. The technique is as follows:

(1) Shake the water sample thoroughly, and transfer, with a sterile pipette, 1 c.c. of the sample to a bottle containing 99 c.c. of *sterile* water. (This is called a *water blank.*) The sample is thus diluted 1:100. If 1 c.c. of this thoroughly mixed dilution is added to another 99 c.c. water blank, this will give a dilution of 1:1000. As many dilutions as are necessary (depending upon the number of living organisms in the original sample) are made in this way.

(2) Place 1 c.c. of each dilution in the bottom of a separate sterile Petri dish. Mark the dishes accordingly. Then pour melted agar, cooled to about 45° C., into each dish, and mix it thoroughly with the water by gently tilting and rotating the dish. It is best to make these plates in duplicate or triplicate.

(3) Allow the agar to harden. Then invert the dishes and incubate them for twenty-four to forty-eight hours.

(4) Discard those plates which contain more than 300 colonies,

because a count based upon them could not be accurate. Count the colonies in each of the other plates. Each colony is assumed to represent a single organism in the original water sample. Multiply this individual plate count in each case by the dilution of the sample in that plate, so as to get the number in 1 c.c. of the original sample. Take the average of the individual plate counts as the final figure.

CHAPTER XVI

COLLECTION OF SPECIMENS FOR BACTERIOLOGICAL EXAMINATION

GENERAL DIRECTIONS FOR THE COLLECTION AND CARE OF SPECIMENS

THE LABORATORY examinations of pus, urine, feces, sputum, or other material collected from the living patient, is relied upon to furnish the final diagnosis, and often to determine or control the treatment, of many of the most common diseases. A bacteriological study of material secured from the dead body at autopsy often serves to explain the cause of death.

The value of the laboratory examination of any material is in large part determined by the care and intelligence with which the specimen is collected. If the proper technique is not employed at the time of collection, or if the specimen is not properly cared for after collection, an examination of it may be impossible or useless. It is frequently desirable to reach a diagnosis in as brief a time as possible in order to permit the proper treatment to be given, and delay or error in diagnosis, by reason of an improperly collected specimen, may be a very serious matter.

General rules. (1) *Secure an uncontaminated specimen.* Avoid contamination of the specimen with organisms from other sources. Use a *sterile container,* and protect the specimen from contamination with dust bacteria as you would any culture tube. When cultures or smears are made directly from an infected part of the body, *sterile swabs or needles* must be used, and the greatest care must be taken to avoid picking up organisms from adjoining tissues or other sources. This is not in every case an easy matter, but an effort must always be made to secure as nearly pure a specimen as possible.

(2) *Label specimen fully and accurately.* Every specimen, smear, or culture must be fully, clearly, and accurately labeled.

The label should indicate unmistakably the patient from whom the material was obtained, the date, the nature and source of the specimen, and the kind of examination desired. Special pains should be taken to see that the name attached to a specimen is the proper one, for serious results may follow failure to associate a patient with his own specimen. If a printed form is to accompany the specimen to the laboratory, this must be filled out completely, accurately, and in a legible manner. The bacteriologist is able to save time, to perform a more intelligent examination, and to preserve more accurate records, if the fullest possible information is given him as to the clinical state of the patient, the exact nature of the specimen, the time or the manner in which it was secured, and the kind of studies he is expected to carry out.

(3) *Do not add a disinfectant unless this is especially ordered.* As a rule disinfectants are not to be added to a specimen intended for bacteriological examination, because the bacteriologist may wish to cultivate the microbes in the specimen or to observe them in a living state. The only exception to this rule is in the case of sputum or feces from a case of suspected tuberculosis. Ordinarily, this material is used solely for making smears. But here also it is best to omit the disinfectant, so that the bacteriologist may make cultures from the specimen if he desires.

(4) *Handle with care to avoid infection.* Since no disinfectant is added, specimens may contain very many living germs in a highly dangerous state. The outside of the specimen bottle or other container should not be soiled, so that it may be handled without contaminating the fingers.

(5) *See that specimens reach the laboratory promptly. Place cultures in a 37.5° C. incubator, and other specimens in a refrigerator, immediately after collection.* It is often very important that the examination be made as soon as possible after collection. The best results are obtained when the bacteriologist brings his swabs and culture media to the bedside, or takes his specimen from the patient directly to the laboratory. Failing this, the most reliable results are secured when the material is studied within one or two hours after collection. A specimen of pus, feces, or sputum is practically useless for cultures when more than four hours old.

No time must be lost in getting cultures to the incubator. The incubator temperature of 37.5° C. (body temperature) will favor the development of the pathogenic types of organisms which are being looked for. But if the culture is permitted to stay long at room temperature, some nonpathogenic types may grow so rapidly that they overwhelm the disease germs.

Specimens of urine, feces, sputum, pus, spinal fluid, and similar material must often be kept for a short time before they can be examined. *During this interval between collection and examination, they must be kept continuously in a refrigerator.* Usually a single kind of pathogenic organism is to be looked for in a specimen—the typhoid bacilli in feces, for example, or pneumococci in sputum. If the specimen is allowed to stand in a warm place, the numerous other kinds of bacteria always present in the material might multiply to such an extent that the germs would be very difficult to find.

COLLECTION OF PARTICULAR SPECIMENS

Pus from wounds and other lesions is usually secured with a sterile cotton swab. Abscesses are sometimes lanced and a sample of the pus collected on sterile gauze or in a sterile test tube. A cotton-plugged sterile capillary pipette may often be used to advantage; its tip may be sealed in the flame before sending it to the laboratory.

Sputum for bacteriological examination should be collected carefully, with attention to a number of details. The mouth should be clean; the patient should rinse it with a mouth wash just prior to collection of the specimen. The specimen must contain true sputum, raised from the deeper air passages by a *cough*, and not merely saliva. The best time to collect the specimen is in the early morning, because patients frequently find it easier to raise sputum at the first expectoration in the morning than at any other time. The sputum must be received in a sterile Petri dish or a clean cup and transferred to a sterile wide-mouthed container. Sputum is examined in cases of pulmonary tuberculosis, pneumonia, influenza, whooping cough, bronchiectasis and lung abscess, and in other forms of acute infection of the deeper parts of the respiratory tract.

Feces are collected in a clean, preferably sterile pan. Portions may be placed in sterile wide-mouthed bottles or large test tubes, making the transfer with sterile sticks or large swabs. Fecal material may be secured from very young infants by inserting a sterilized glass tube into the rectum. The feces are always examined in cases of typhoid and paratyphoid fever, dysentery, cholera, and other diseases of the intestinal tract. Cultures are often made from the feces of healthy persons in order to discover *carriers* of typhoid bacilli and other germs. Feces are examined microscopically for hookworms and other intestinal parasites.

Urine is collected for bacteriological examination in cases of suspected infection of the urinary tract. It also may be examined in typhoid fever and other intestinal diseases, in tuberculosis of the urinogenital organs, and occasionally in pneumonia or septicemia, and other diseases. The external genitals and the lower part of the urethra always contain many bacteria, and these must be avoided in order to secure an uncontaminated specimen of urine. A satisfactory sample may be obtained from males if the glans and meatus are first thoroughly cleansed with soap and water. The first urine voided should be discarded, and only the last portion collected in a sterile, wide-mouthed bottle. From a female, a urine sample satisfactory for bacteriological examination can be secured only by use of a catheter—a small rubber tube inserted into the bladder or ureter. The parts must first be washed with soap and water. The catheter must be sterile, and the first portion of urine should be rejected.

Peritoneal or pleural fluid is collected by inserting a sterile needle through the wall of the abdomen or chest and withdrawing the fluid in a sterile syringe or allowing it to run into sterile test tubes. The skin at the site of the puncture must be disinfected, of course, and every aseptic precaution must be taken as in any operation.

In heart and kidney disorders, and in some other organic disease conditions, there may be an accumulation of very large amounts of sterile fluid in the peritoneal cavity. This condition is called *ascites* and the liquid is called *ascitic fluid.* Similarly, an excess of fluid may accumulate in the pleural or pericardial cavities. *Sterile* fluids of this sort, originating from some cause other than an infection, are called *transudates. Ascitic* fluid, aseptically collected, is used in the bacteriological laboratory as an enrichment for culture media.

Spinal fluid is obtained for examination by a process known as *lumbar puncture.* A large sterile hollow needle or trochar is passed into the spinal canal through a sterilized and anesthetized spot on the skin of the back, slightly to one side of the midline, between the third and fourth lumbar vertebrae. The strictest asepsis is observed throughout this operation and at its completion the puncture wound is protected with a sterile dressing. In pathological conditions the spinal fluid is usually much increased in amount and under much greater pressure than normal, so that it flows readily from the needle. It may be allowed to run directly into sterile centrifuge tubes. When fluid from the same patient is collected in several tubes, these must be numbered to show in what order the specimens were obtained. Often blood appears in the first drops of fluid collected, while later, unmixed spinal fluid is secured.

The spinal fluid is normally a clear liquid, containing only a few leukocytes, and *sterile.* Therefore, if bacteria, or an unusual number of pus cells, are found in it, this is evidence of a diseased condition. The fluid may still appear clear and yet be very abnormal. In syphilis and infantile paralysis (poliomyelitis), it is usually clear; and in tuberculous meningitis, it is either clear or slightly cloudy. In cases of acute meningitis, however, due to the *Streptococcus*, *Pneumococcus*, *Meningococcus* or other bacteria, the fluid is usually very cloudy and contains many pus cells.

Spinal fluid from cases of syphilis is not used for cultures or smears, but only for a Wassermann or other diagnostic test. The sample should be sterile.

Blood. Many important facts are learned from examination of the patient's blood. The blood may be collected for study of the blood cells, or for tests on the blood-serum, or for smears or cultures to detect micro-organisms that may be in it. There are two common methods of securing blood for examination. These are: (1) the skin puncture method, by which a few drops of blood are secured from a puncture wound of the finger or ear, and (2) the venous puncture method, by which a considerable amount is obtained through a hollow needle inserted into a vein.

Technique of skin puncture method. The lower lobe of the ear or tip of the finger is prepared for puncture by cleaning thoroughly with an alcohol sponge. An increased flow of blood to the fingers may be secured by shaking or swinging the hand vigorously, and the ear may be rubbed gently. After the alcohol has dried, the skin is then punctured, *not cut,* with a quick determined movement. A special blood lancet may be used for the puncture, but a sharpened, stiff needle or pin, or half of an ordinary writing pen, will serve. The puncture needle must be sterile. The blood should well out of the puncture wound spontaneously. It is best to avoid squeezing the ear or finger in order to secure enough blood. When the bleeding process is completed, the puncture wound is wiped with an alcohol sponge or touched with tincture of iodine.

Use of skin puncture method. This way of obtaining drops of blood serves *for smears* to be examined for malaria parasites and other organisms.

Also this method is used to secure blood for a determination of the number of red blood cells ("red count"), or white blood cells ("white count"), and for making smears from which to determine the relative proportion of the various types of white blood cells ("differential count"). The amount of hemoglobin, the coagulation time, and other properties of the blood may be tested also.

The cell counts are made by drawing up a small amount of blood from the drop on the finger or ear into special blood counting pipettes, which accurately measure and dilute the blood. Drops of this diluted blood are examined in special chambers under the microscope and an actual count is made of the number of red or white blood cells in a known amount of blood. From this, an estimate is made of the total number of the cells in a cubic millimeter of the patient's blood. The differential count is made by counting the first 200 white blood cells seen in a stained smear of the blood and classifying them into the various types—the polymorphonuclear leukocytes, lymphocytes, and so forth. The percentage of each type present is then determined. This differential count and the white count are very important in cases of germ disease, because they serve as an index of the severity of the disease and of the amount of resistance the patient has.

Technique of venous puncture method. In adults the veins at the bend of the elbow are used (Fig. 49). A bandage of cloth or a tourniquet of soft rubber tubing is applied to the upper arm and the patient is made to clench the fist. This makes the veins at the elbow and lower arm stand out prominently. The skin about the veins is cleansed with soap and water (if necessary), then washed with 70 per cent alcohol. Tincture of iodine may be used. A sterile hypodermic needle is then inserted into the vein and the blood is caught in a sterile tube. When the bleeding is complete, the tourniquet is immediately removed and an alcohol sponge is pressed firmly against the puncture wound until the bleeding stops.

When it is necessary to secure blood from an infant, for the Wassermann or other tests, the skin of the finger or heel may be punctured, or the blood may be secured from the large vein that lies just below the anterior fontanel (the soft spot on top of the head). This area is shaved and cleansed, then a sterile needle is thrust into this vein and the blood withdrawn into a syringe.

Use of venous puncture method. This method is used whenever considerable amounts of blood are required for chemical or bacteriological tests upon the *blood serum.* Blood for the *Wassermann test* (for syphilis) is always secured in this way. The blood is allowed to *clot* in the sterile collection tube, and the clear serum which separates off from the clot is used for the test. The specimen should be sterile, and must be kept in a refrigerator until the test is made.

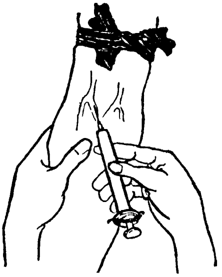

FIG. 49. Drawing showing method of bleeding from the veins at the bend of the elbow by use of a hypodermic syringe. (Redrawn from Todd and Sanford.)

CULTURES AND SMEARS FROM THE LIVING PATIENT

Blood cultures. *The blood of healthy persons is sterile.* But in various diseases living bacteria may be present in it and cultures of the blood serve as one of the most important methods of diagnosis. Usually 10 c.c. or 15 c.c. are withdrawn with a syringe from the arm vein as described above. *The blood is at once expelled into a flask of sterile broth.* Bile broth is used when typhoid bacilli are likely to be present in the blood, and brain broth when the presence of streptococci, pneumococci, meningococci, or other organisms is suspected. Some of the blood may be added to melted agar and the agar poured into Petri dishes. If the blood must be carried some distance to the laboratory before it can be inoculated into media, it can be caught in a flask containing a sterile, 10 per cent solution of sodium citrate. Enough of this solution should be used to give a final concentration of about 0.1 per cent of sodium citrate after mixture with the blood. This will be sufficient to prevent clotting.

Cultures from the nose and throat. Material for nose cultures is secured by passing a small, sterile, cotton swab straight back through the nostril. The swab must not be large, or loose upon the stick, otherwise it may catch in the nose.

Cultures from the throat are made by touching a sterile swab to the *diseased part only*. There must be plenty of light in the throat so that it is possible to see exactly what is being done. When the presence of diphtheria bacilli in the nose or throat is suspected, the medium used must be Loeffler's serum medium. For all other purposes, blood agar is the most suitable medium.

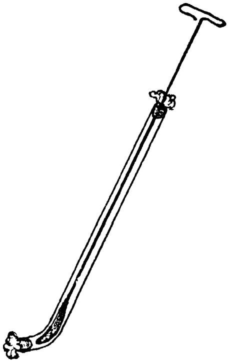

Fig. 50. A West tube for making cultures from the nasopharynx. The cotton plug at the curved end of the glass tube is removed, and this end of the tube is passed into the mouth and back of the uvula. The swab is then pushed out, and rubbed over the nasopharynx, then withdrawn into the tube before removal from the mouth.

Cultures from the nasopharynx. The nasopharynx is the upper part of the throat back of the soft palate. Cultures from this region are made by use of a special swab (Fig. 50). The cotton is wrapped about the end of a wire having a nearly right angle bend, which is enclosed in a glass tube similarly bent. The tube is introduced into the mouth and the curved end passed back of the soft palate. The swab is then pushed out and rubbed across the nasopharynx, then withdrawn into the tube before it is removed from the mouth.

Other cultures. Cultures from the *eyes* may be made by rubbing a small sterile cotton swab wet with sterile salt solution very gently over the surface of the conjunctiva.

Cultures from the *cervix or uterus* can be made only when the parts are exposed by the use of a sterile speculum. The very greatest care is required not to contaminate such cultures with organisms from the vagina.

Direct smears. There is frequently more to be learned from a smear made directly from an exudate or lesion than from cultures of the same material. Smears alone are relied upon for the diagnosis in cases of acute gonorrhea, Vincent's angina, and in some other diseases. Often the examination of smears from the pus of abscesses, wounds, inflamed eyes or ears, and other lesions, gives important clues as to the cause of the infection. Smears must be *thin*, and of good size. The slide must be clean and not greasy. When a smear is to be made with a swab, ***roll*** the swab over the slide and avoid unnecessary pressure, so that the pus cells will not be broken up.

REVIEW QUESTIONS—CHAPTER XVI

1. Give five rules for the collection and care of specimens.
2. Explain why it is important to have specimens (*a*) uncontaminated, (*b*) carefully and fully labelled, (*c*) without disinfectant. Why must the specimen be handled carefully? Why must cultures be placed at once in an incubator? Why must other specimens be placed in the refrigerator?
3. How might the pus from an abscess or wound be collected?
4. What is meant by "true" sputum? How should it be secured for examination?
5. How might a sample of feces be obtained?
6. Why must especial care be taken in securing a specimen of urine for bacteriological examination? Give a method for collecting urine for this purpose from (*a*) males, (*b*) females.
7. Define: *peritoneal fluid*, *pleural fluid*, *ascites*, *ascitic fluid*, *transudate*.
8. How is sterile ascitic fluid obtained for use in bacteriological culture media?
9. Explain how spinal fluid is collected by lumbar puncture. How does it appear in disease conditions?
11. Name two methods of securing samples of blood.
12. Give a practical method of securing blood for each of the following purposes: (*a*) smears for diagnosis of malaria and other diseases, (*b*) blood cell counts, (*c*) Wassermann test and other serological tests.
13. How is a blood culture made?
14. Outline method of obtaining cultures from the nose, throat, nasopharynx, eyes, cervix, and uterus.
15. What value have smears made directly from the diseased tissues or exudates? Name two diseases commonly diagnosed on basis of smears only. Mention some details of technique which help to make a good smear.

PART THREE

METHODS OF DESTROYING MICRO-ORGANISMS AND OF CONTROLLING THE SPREAD OF COMMUNICABLE DISEASES.

SOURCES AND MODES OF INFECTION

CHAPTER XVII

DESTRUCTION OF MICRO-ORGANISMS BY PHYSICAL METHODS

IN PREVIOUS chapters we have discussed the effect of environment upon the life of microbes and have pointed out their sensitiveness to drying, sunlight, cold, heat, and other physical and chemical conditions. We shall now consider in this and the following chapters the practical ways in which micro-organisms may be destroyed by deliberately exposing them to these harmful influences, and how the spread of germ diseases may thus be prevented. But first it will be necessary to define the various terms which are used in connection with this subject.

Sterilization. By *sterilization* is meant the *complete destruction of all micro-organisms.* An object which is quite free of any living microbe is said to be *sterile.* The meaning of these terms sterile and sterilization is absolute; there is no such thing as a "practically sterile" or "nearly sterile" object. A thing is either sterile, or it is not sterile.

Disinfection. *Disinfection* means the *destruction of pathogenic micro-organisms.* The term *disinfectant* is applied to an agent, usually a chemical, which is *used to destroy disease-producing organisms.* Of course, it may kill harmless bacteria, too, but it happens that disease germs are, in general, more easily destroyed than the harmless saprophytic types, so that disinfection may be accomplished in many instances with agents that do not truly sterilize. Thus, the feces of a typhoid patient may be successfully disinfected with a chemical which kills the typhoid germs, even though it does not destroy all the bacteria present.

Germicide. Any agent that *kills* micro-organisms may be called a germicide.

Bacteriostasis. This refers to a condition in which bacteria are *prevented from multiplying,* though not killed. Low temperatures, weak concentrations of germicides, and certain dyes, for example, may keep bacteria in a state of suspended animation, and so are said to have a bacteriostatic effect.

Antisepsis. This term and the more widely employed derivative *antiseptic* cannot be defined very satisfactorily. Literally, an antiseptic is a substance that opposes *sepsis*, a word derived from the Greek meaning rotting, putrefaction, decay. Since it is the growth of micro-organisms that causes sepsis, an antiseptic must have the property of *preventing the multiplication of microbes*, or, in other words, it must have a bacteriostatic effect. It may be a much weaker agent than a disinfectant, for the latter actually destroys germs.

Unfortunately most laymen and many doctors do not use *antiseptic* in this strict and literal sense, but give it the same meaning as disinfectant. In consequence, an "antiseptic" in current usage may refer to a truly germicidal agent in one case, or to a substance that has merely a bacteriostatic action in another case. It is best for professional people to employ the term only in its literal sense to mean an agent that inhibits the growth of microbes, without destroying them.

PHYSICAL METHODS OF STERILIZATION AND DISINFECTION

Drying. There is considerable difference in the resistance to *partial* drying, shown by the different species of bacteria. For example, the staphylococci on the human skin, most of the saprophytic organisms common in the soil and dust, the germ of diphtheria, and particularly the bacillus of tuberculosis can withstand a comparatively high degree of partial drying, but the vast majority of parasitic and pathogenic bacteria die very quickly when deprived of an abundance of moisture. Spores are much more resistant to drying than vegetative cells. Practically, drying as a method of destroying bacteria is usually combined with the action of sunlight or heat, with the result that all types of bacterial cells are completely dried out and quickly destroyed. The preservation of foods by drying is one important application of this method of killing microbes.

Sunlight and ultraviolet light. Direct sunlight has a powerful germicidal action. Exposure to direct sunlight for a sufficient time will kill spores as well as vegetative cells. Tuberculosis germs are killed in a few hours. It is often possible to make deliberate use of this germ-killing power of sunlight for the disinfection of clothing, bedding, mattresses, and other materials. The germicidal property of the sunlight is not due to the ordinary light which we see, but to the very short, invisible, light rays beyond the violet end of the spectrum—the ultraviolet rays. It should be remembered that these rays are filtered out by ordinary glass, and that the sun must shine directly upon an object in order to exert its germicidal effect.

Ultraviolet rays are easily produced artificially by passing an electric current through vaporized mercury in quartz tubes. The rays pass through the tubes of quartz and so can be applied to any object. They destroy bacteria in a few seconds or minutes; molds and yeasts are somewhat more resistant. Ultraviolet light has been employed for purification of drinking water and swimming pool water, but it is not very effective for such purposes and is too expensive to justify wide use.

Concentrated solutions. It has already been mentioned that foods may be preserved by immersing them in strong salt or sugar solutions. This is due to the fact that in such concentrated solutions the bacterial cells lose water through osmosis and so shrink and die.

Cold. Cold prevents the multiplication of the ordinary bacteria, and refrigeration serves as an admirable method for the preservation of food and other substances which are easily decomposed by micro-organisms. Some of the common disease germs, such as the organisms causing meningitis, gonorrhea, and syphilis are actually killed by cold. But most bacteria are not destroyed even at very low temperatures. The bacillus of typhoid fever, for example, may be frozen in a block of ice and still be able to grow when carefully thawed out and returned to a favorable temperature.

Heat. The application of heat in one form or another is the most widely used method of destroying bacteria. It will be remembered that the thermal death point of the vegetative cells of most of the ordinary bacteria is about 65° C. (or about 145° F.), but that spores and some unusual organisms are very much more resistant to heat. For this reason, if we wish to sterilize an object truly, high temperatures continued for considerable periods of time must be applied.

Practical methods of sterilization by heat.

Heating in the open flame. Heating small glass or metal objects by holding them in the free flame, as in the Bunsen burner, is an effective method of sterilizing them. This is the way the bacteriologist sterilizes his inoculating needle and the glass capillary pipettes he sometimes uses. The method may be used in an emergency for sterilizing a strip of adhesive tape or the tips of forceps or other instruments.

The inside of a medicine glass may be sterilized by first wiping it out with alcohol or ether and then touching a flame to the adherent liquid; the fluid will burn and leave the glass sterile. A small instrument might be sterilized in a similar way.

Burning or incineration. Burning is a safe and cheap method of disposal of small objects of no value, such as used swabs, or dressings, or paper sputum cups. Whenever possible, incineration of articles contaminated with disease germs is to be recommended.

Exposure to hot air (dry heat oven). In bacteriological laboratories, one of the most commonly used pieces of apparatus is a sterilizer called the *dry heat,* or hot air, *oven* (Fig. 51). This oven is similar to the ordinary baking oven used in the home. It is a double-walled chamber heated by gas or electricity and so constructed that it will stand a very high temperature. This apparatus is used in the laboratory almost entirely *for the sterilization of test-tubes, flasks, Petri dishes, and other kinds of glassware.* An exposure of at least one hour at a temperature of about 160° C. is necessary to assure the complete sterilization of objects in the dry heat oven. *As a routine method, glassware is held in the oven at 170° C. for one and one half hours.* Cotton and paper may be charred if the temperature runs over 180° C.; this must be avoided. After the period of heating, the oven must not be opened until the temperature has fallen to 100° C. or less, because too sudden cooling of the glassware may crack it.

Boiling. Boiling is such a commonplace, everyday procedure that its use is sometimes neglected for a more complicated method. Boiling water has a temperature of 100° C. (212° F). The vegetative forms of most bacteria are killed by a few minutes exposure to this temperature, but it must be remembered that spores may resist boiling for as long as one hour or more.

The time required to sterilize by boiling will vary a great deal with the nature of the material. It must be remembered that, in order for an object to be sterilized, it *must be completely immersed in the boiling water* and the boiling must be continued long enough for all parts of the object to reach the sterilizing temperature. Boiling is especially useful for the disinfection of dishes and tableware, handkerchiefs, bed linen, bedclothes, and other things contaminated by patients, and for the sterilization of surgical instruments, hypodermic needles, and syringes.

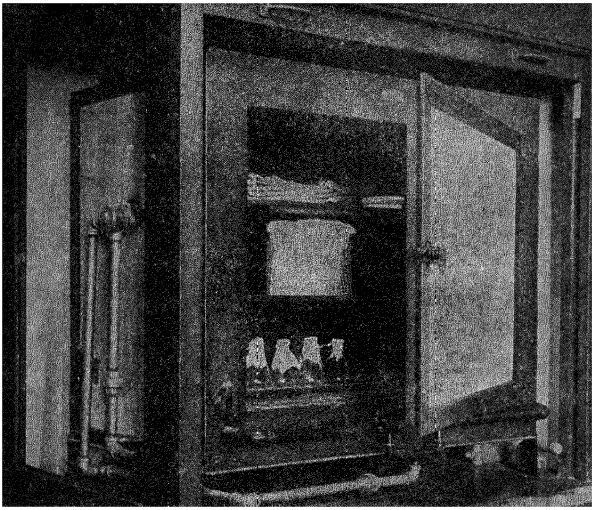

FIG. 51. A dry heat oven, showing types of laboratory glassware which are sterilized in this apparatus.

Heating in flowing steam. Live steam is one of the most effective sterilizing agents. Steam kills micro-organisms much more rapidly than hot air; the protoplasm coagulates and dies more quickly. Also steam penetrates substances readily, carrying its heat along with it.

Flowing steam, that is, steam which is not confined under pressure, has the same temperature as boiling water, or 100° C., and never rises above this temperature. For this reason, flowing steam is not effective against bacterial spores unless it is applied for a very long time. In practice a method of intermittent exposure to flowing steam, called the *fractional or discontinuous method of steam sterilization,* is employed. In this method the material to be sterilized is exposed to the steam for half-hour periods on each of three to five successive days. The apparatus used in the laboratory is called the *Arnold sterilizer.* An ordinary double-boiler or steamer might be used at home. At the first heating, most of the bacteria in the active vegetative stage are killed, but not the spores. Then, in the interval between the first and the second heating, the spores which survived the first heating germinate into vegetative cells. These should be killed the second day, but in order to sterilize the material surely, the process of intermittent heating is usually continued for another day or two.

The method is somewhat uncertain at the best, and in most laboratories at the present time the Arnold sterilizer is not often used, except for the sterilization of certain solutions or culture media which would be altered in some undesirable way by temperatures above 100° C.

Heating in steam under pressure. When steam is confined under pressure in a closed chamber, it is a much more effective sterilizing agent than flowing steam, because it penetrates better and particularly *because it attains a higher temperature.* It is the temperature, not the pressure which is the more important. The relation between the pressure of steam and the temperature is shown in the following table.

Pounds of pressure of steam	*Temperature*	
(Above atmospheric pressure)	Centigrade	Fahrenheit
0	100°	212°
5	109°	228°
10	115.5°	240°
15	121.5°	251°
20	126.5°	260°

The autoclave or steam pressure sterilizer. Sterilization by steam under pressure is carried out in laboratories and hospitals in an apparatus called an *autoclave* (Fig. 52). The "dressing sterilizer" of the hospital is an autoclave. The pressure cookers used at home for cooking or canning of food are small autoclaves. So also are the large retorts used in canning factories for sterilization of tin cans of food. The autoclave is essentially a double-walled metal chamber fitted with a door which can be closed very tightly. There is a safety valve to permit the escape of the steam should the pressure get too high. There are usually two gauges, one to show pressure of steam in the outer jacket and another to show the pressure in the inner chamber. There are valves to hold the steam in the jacket and to send it into the sterilizing chamber. There is an exhaust valve to let the steam escape from the inner chamber. There should also be a thermometer to show the temperature within the chamber, but some autoclaves are made without thermometers. The steam is often generated by a gas-heated boiler arranged beneath the autoclave, though in some large laboratories and hospitals steam from the main boiler room is piped directly to the apparatus.

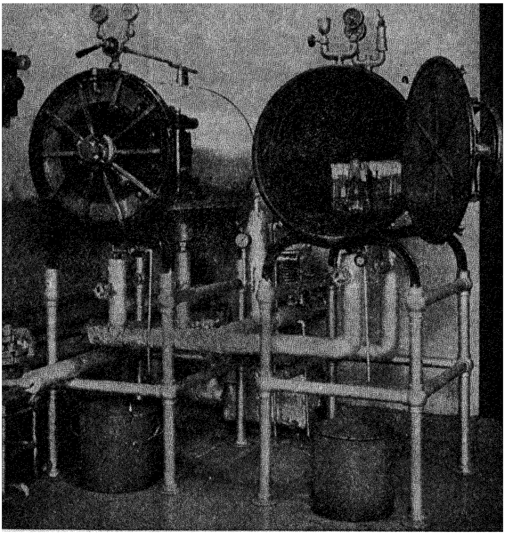

FIG. 52. Autoclaves (steam pressure sterilizers). The steam for the autoclaves shown here is supplied directly from the main steam line.

Articles to be sterilized in the autoclave are usually exposed to a pressure of *15 pounds of steam* (that is, to a temperature of about 121° C.) *for twenty to thirty minutes.* The time required depends upon how bulky the material is, larger articles naturally requiring longer to heat through, and also how closely the material is packed into the chamber. In order to be sterilized, *every part of the material must be heated to the sterilizing temperature for the necessary length of time.*

Both the hospital and the laboratory find constant use for the autoclave; in the hospital for the sterilization of sponges and dressings and other materials for the operating rooms and wards, and in the laboratory for bacteriological culture media and many other things.

Practical points concerning the operation of the autoclave. The efficient operation of the autoclave is a very important matter, particularly in hospitals where this apparatus is depended upon to sterilize the dressings and other materials used in surgical and obstetrical work. Often nurses have the responsibility of operating the hospital autoclaves.

The following are the essential steps and precautions to be taken in using any autoclave:

(1) If the steam must be generated by heating water, see that there is plenty of water in the boiler before turning on the heat.

(2) When placing articles to be sterilized in the chamber, leave room so that the steam can pass around and about each object. *Do not pack articles too close together and do not jam the autoclave full.* This is probably the commonest mistake made in operating hospital sterilizers. It amounts to taking a serious and unnecessary risk, for when an autoclave is packed too closely and too full, there is no certainty that all articles will be heated enough to be sterilized. It is better to place articles to be sterilized in many small containers than in a few large ones. Close and seal the door as tightly as possible.

(3) Allow the steam to enter the *jacket* until the gauge shows a pressure of about 15 pounds. Keep the valve controlling the exhaust from the inner chamber open, so that the air in the chamber may escape. Now allow the steam to enter the inner chamber by manipulating the proper valve, meanwhile leave the exhaust valve open. Allow the steam to run in for several minutes with the exhaust open, until nothing but dry steam comes out of the exhaust. This is necessary because *all the air in the chamber must be driven out and entirely replaced by steam.* Otherwise, although the gauge might show a pressure of 15 pounds, it would not be 15 pounds of steam, but a mixture of steam and air, and this mixture could not be hot enough to sterilize. When all the

air has thus been driven out and only steam comes through the exhaust, close the exhaust valve almost but not quite completely, so that a small amount of steam may escape from it throughout the sterilization process. When the valve is nearly closed, the pressure of steam in the chamber will quickly reach the required 15 pounds. The gas under the boiler may be lowered or the steam supply shut off enough so that the 15 pounds pressure is constantly maintained.

(4) Note the time when the steam in the chamber reaches the pressure of 15 pounds and maintain this pressure continuously for the next 20 to 30 minutes.

(5) At the end of this period turn off the gas under the boiler, or shut off the steam supply and allow the autoclave to cool down. When only linens, dressings or similar materials are being sterilized, the exhaust valve may be opened to allow the steam to escape quickly; but if any liquids are present in tubes or flasks, the exhaust valve must not be opened at all until the pressure has returned to 0, for otherwise the liquids will boil violently and cotton plugs will be blown out. Some autoclaves are made so that a slight vacuum (4 or 5 pounds) may be produced in the chamber before the exhaust valve and door are opened and the articles removed. This helps to make the articles dry, but it is not necessary, for if the autoclave is running properly, cotton plugs, dressing, and other objects will come out of the autoclave hot and quite dry.

It is a practice in many hospitals to place in each load of material to be sterilized a "sterilizer control" or indicator. This is a small glass tube, with a string attached, containing a chemical which changes color at the sterilizing temperature. It should be placed in the center of a large package in the midst of other articles. After sterilization, the tube may be pulled out by its attached string and examined. If the expected color change has occurred, this indicates that the sterilizing temperature was attained at least for a short time, and it is certain proof that the particular package from which the tube was drawn has been through the sterilizer. Another method often employed is to place in the autoclave a thermometer which will show the highest temperature reached. Too much dependence should not be placed upon these controls, however. They do not show whether the necessary temperature was constantly maintained for a sufficient length of time. The only safe and proper way is to operate the autoclave most carefully at all times, observing all the precautions outlined above.

Pasteurization. This is a special method of applying a mild degree of heat to milk and other liquids for destroying undesirable and harmful nonspore-forming microbes. It is really not a process of sterilization, but rather of *disinfection.* It was originated by Louis Pasteur and used by him for killing microorganisms which cause undesirable fermentations in the process of making wine.

Pasteurization is now extensively used for freeing *milk* from disease germs (Fig. 56). *The process of pasteurization of milk consists of heating it to 60°–65° C., (140°–150° F.) for one half hour, followed by a rapid cooling.* In most modern dairies the raw milk is carried in pipes from large receiving tanks to the pasteurization machines. Just before entering these machines, it is passed through hot pipes which warm it to the pasteurizing temperature. The pasteurizers consist essentially of tanks in which the milk is held at the necessary temperature (usually 142° F. is used) and continuously stirred for half an hour. Then the milk passes through long cold pipes, in which it is rapidly cooled, and from these pipes is carried to automatic bottling and capping machines. As we have already learned, this heating destroys the vegetative forms of practically all bacteria, including those of any disease germs that may be present. The milk is not sterilized —pasteurized milk will sour, though more slowly and in a manner somewhat different than unheated milk—but all pathogenic bacteria or viruses likely to be found in it are killed.

Pasteurization does not appreciably alter the quality or flavor of milk, although its property of preventing scurvy is somewhat diminished. Infants need the antiscorbutic principle, but this is readily supplied from other foods, and boiled or pasteurized milk is the only proper kind of milk for them. Pasteurization of all milk is encouraged by health authorities everywhere and is required by law in many localities, for it is one of the most important measures for the public health.

Filtration. This is a method of making liquids or gases sterile by permitting them to *filter* through the appropriate material.

Air filters. In the laboratory most of our test tubes and flasks are stoppered with a plug of cotton. Air and other gases pass freely in or out of the tubes through the cotton, but microorganisms in the dusty air are caught in the meshes of the cotton and cannot get through into the tube (unless the cotton is wet). Therefore, the cotton plugs serve as efficient filters.

Filtration of liquids. Filters for making *liquids* free of any visible or cultivable microbes have very important uses in micro-

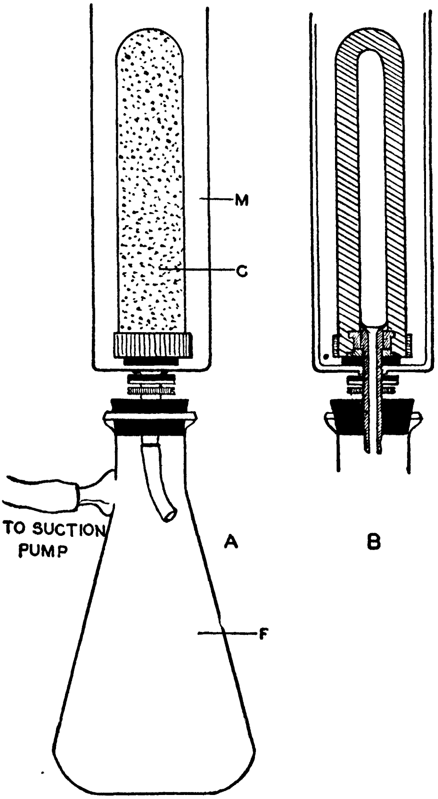

Fig. 53. A, a Mandler filter set up for use. A glass test tube of larger diameter than the filter candle (C) is inverted over the candle (this tube is not shown in the drawing), then the culture or other material to be filtered is poured into the glass mantle (M). The fluid rises inside the inverted tube so that it covers the entire candle. Then as suction is applied the fluid is drawn through the filter while the ordinary bacteria are held back, and a clear filtrate, free of any visible microbes, flows down into the sterile flask (F) below. B, section through the filter, showing the hollow construction of the filter candle.

biology. There are several types of laboratory filters; those most commonly employed are the Berkefeld and Mandler filters. They are made of diatomaceous earth pressed into the shape of a hollow candle. The filter candle and a suction flask to receive the filtrate are sterilized, usually in the autoclave, and then carefully fitted together. The liquid to be filtered is poured into the glass "mantle" about the filter candle, suction is applied, and the liquid is drawn slowly through the porous filter and falls into the sterile flask below. As the fluid passes through the filter candle, all the ordinary micro-organisms in it are held back, and the filtrate is bacteriologically sterile, that is, free of any microbe that can be cultivated on lifeless media (Fig. 53).

Filters are used in the laboratory to sterilize blood-serum, ascitic fluid, sugar solutions, or other liquids which cannot be sterilized by heat without altering them in some undesired way. Also *exotoxins*, enzymes, and other products of the growth of bacteria are separated from the organisms which produce them by use of these filters. Finally, the *filtrable viruses* are isolated from the body tissues or fluids which contain them, and separated from ordinary microbes, by means of these filters.

Filtration of drinking water. In most communities, filtration through sand and gravel is one of the most important measures used for the purification of the public water supply (Fig. 55). In this kind of filtration there is more than a mechanical straining. On the surface of the sand filter a scum develops which consists largely of putrefactive micro-organisms, and as the water passes slowly through the sand, most of the contained bacteria are held back in this surface scum, and there destroyed in competition with the hardy putrefactive forms. Properly filtered water is nearly free of all bacteria and quite free of dangerous germs.

REVIEW QUESTIONS—CHAPTER XVII

1. Define: *sterilization, sterile, disinfectant, disinfection, germicide, bacteriostasis, antiseptic.*
2. Why is drying a possible method of sterilization or disinfection? Discuss its practical use.
3. What is the effect of sunlight and ultraviolet light on bacteria? Discuss the practical use of these agents.
4. What is the effect of concentrated solutions on bacteria? How can this be put to practical use?

5. What practical value has cold as means of controlling bacterial life?
6. What is the approximate thermal death point of the vegetative cells of most bacteria? Why must higher temperatures be used in the practice of sterilization?
7. Give examples of the practical use of the open flame and burning for sterilization purposes.
8. Describe the dry heat oven. For what is it used? Give the essential details for the successful operation of the oven.
9. Give practical examples of the use of boiling as a means of sterilization. What factors bear upon the success of the method?
10. Describe the Arnold sterilizer. What is the temperature of flowing steam? What is the discontinuous method of sterilization, and what is the principle underlying it?
11. What happens to the temperature when steam is confined under pressure? What is the temperature of steam under 5, 10, 15, and 20 pounds pressure? Which is the more important in bringing about sterilization, the pressure or the temperature of the steam?
12. Describe an autoclave.
13. What is the usual time and pressure of steam required for sterilization of articles in the autoclave?
14. Describe five steps in the operation of an autoclave and discuss the precautions necessary for success.
15. Why is it important not to pack articles too closely into the autoclave? In general, what is the relation between the amount and bulk of material in the autoclave and the time required to sterilize it?
16. Why is it necessary to replace all the air in the chamber with steam?
17. Why is it necessary to allow the autoclave to cool off without opening the exhaust valve when liquids in tubes or flasks are being sterilized?
18. Discuss the practical value and limitations of sterilizer controls or indicators.
19. Define *pasteurization.* What is the principal use of the method at present? Why is pasteurization of milk an important public health measure?
20. How do cotton plugs serve as filters to keep out microbes?
21. Describe the construction and operation of a Berkefeld and Mandler filter. Give examples of the practical use of such filters in bacteriological work.
22. How is filtration used for the purification of water? What other factors besides simple mechanical filtration account for the result?

CHAPTER XVIII

DESTRUCTION OF MICRO-ORGANISMS BY CHEMICAL GERMICIDES

A LARGE number of chemical substances may be employed as germicides and many new disinfectants are being added to the list continually. Comparatively few, however, are sufficiently cheap and effective to deserve wide use. Some germicides may be safely used within the body for the treatment of infections of the urinary bladder, kidneys, and other internal organs, but the great majority of powerful disinfectants are almost as poisonous to the human tissues as to bacterial cells. Unnecessary irritation of the tissues by chemicals is to be avoided, for this may only delay healing. Consequently, most disinfectants are of little value in treatment. They are most useful, however, in *preventing* infections, particularly for disinfection of the skin before an operation, or a hypodermic injection. They are of especial value for the disinfection of the feces, sputum, and other germ-laden discharges from a patient with a communicable disease.

Factors affecting the action of chemical germicides. Just how chemicals act to kill micro-organisms is not known; the end result is probably a coagulation of the protoplasm of the cells. The process of killing germs by a disinfectant may be thought of as a chemical reaction between the bacterial cells and the active parts of the disinfectant. A number of factors influence the speed and completeness of this reaction, and these must be understood in order to use disinfectants intelligently. There are three fundamental factors which affect the action of any chemical germicide. These are: (1) the concentration of the active chemical, (2) the time allowed for disinfection, and (3) the nature of the material to be disinfected.

The germicidal power of a chemical naturally differs according to the strength or concentration in which it is applied. A disinfectant may have no effect upon bacteria in a very weak solution, or it may actually stimulate their growth. In somewhat greater concentration the same chemical may have an antiseptic or bacteriostatic action, that is, it may prevent the growth of germs, and a still stronger concentration of the same reagent may be an excellent disinfectant, killing germs very readily. The presence of water is necessary for disinfection, and disinfectants, in general, are most active in a watery solution. But *this solution must be of sufficient concentration and it must be used in sufficient amounts* to be effective as a germicide. It is well to remember that a liquid disinfectant is always more or less diluted when mixed with the material to be disinfected. Thus, if one pint of a 5 per cent lysol solution is mixed with a pint of feces, its strength is reduced to that of a 2.5 per cent solution.

The commonest mistake made in the use of disinfectants is the failure to allow sufficient *time* for them to act. The effect of chlorine and a few other germicidal chemicals is almost instantaneous, but these are the exceptions to the general rule. The great majority of disinfectants do not act quickly and in practice *it is very important that sufficient time be allowed for complete disinfection of any material.* The germicidal agent must be given time to penetrate all parts of the material, because the active chemical must reach the germs themselves in order to kill them. To disinfect a mass of feces, for example, at least an hour or more is required even if an excess of a powerful germicide is used.

The action of a disinfectant varies with the character of the material acted upon. Organic matter interferes more or less with the germicidal action of any chemical and some disinfectants are of no value in the presence of abundant organic material. Bichloride of mercury, for example, cannot be relied upon to disinfect sputum or feces because it combines with the albuminous matter and may not reach the bacteria at all. *The proper disinfectant must be chosen according to the nature of the material to be disinfected.*

Measurement of the germicidal power of chemicals. It is possible to measure the activity of disinfectants with a considerable degree of accuracy by certain laboratory procedures. A number of different tests are used, but in principle they are all alike. The action of pure phenol (carbolic acid) on pure cultures of *Eberthella typhi*, *Staphylococcus*, or other bacteria, is taken as the standard, and the relative germicidal value of other disinfectants is determined by comparing their germ-killing power with that of phenol under the same conditions. Definite dilutions of phenol and of the disinfectant to be standardized are used so that the result of the test can be expressed by a number. This number is called the *phenol coefficient.* The coefficient of phenol itself is 1.0. If a disinfectant is less active than phenol in killing germs under the same conditions, its phenol coefficient is some figure less than 1.0; if it is more active than phenol, its phenol coefficient will be greater than 1.0.

While these tests are useful, they do not give a complete picture of the germicidal power of any substance. In order to discover the full potentialities, each disinfectant would have to be tested repeatedly against many different organisms, under a variety of conditions, and especially in circumstances simulating as closely as possible those prevailing where it may actually be used. It would then be found that for each disinfectant there are certain zones of concentrations within which it is, in turn: (1) without effect, (2) stimulating to the growth of the test organisms, (3) bacteriostatic, (4) definitely germicidal in a useful way, and (5) too concentrated to be of practical value. Few disinfectants have been so thoroughly studied, and at present, our information about their germicidal power is based almost entirely on rather crude trial-and-error tests.

Principal disinfectants for general use. The most reliable and most widely used disinfectants for general purposes are; (1) carbolic acid, (2) cresol, lysol, and similar compounds, (3) formalin, (4) chlorine and its compounds, and (5) lime.

(1) *Carbolic acid (phenol).* This is the germicide used by Lister in the epoch-making work which introduced antiseptic methods into surgery. It is employed, as described above, as a standard for determining the relative strength of other disinfectants. Carbolic acid is a "coal-tar" compound, that is, one of the products which results from the destructive distillation of coal. In the crude form, it is a mixture of *phenol* (pure carbolic acid) and similar compounds and has a high germicidal power. Pure phenol comes in the form of white crystals. These melt with a little warming and readily take up water to form a brownish liquid. Solutions are most easily made in hot water. The pure

carbolic acid is a somewhat less powerful germicide than the crude product.

A 5 per cent solution of either the crude or the pure chemical is the strength usually employed. This solution is an effective germicide even in the presence of organic matter and may be used for disinfecting feces or sputum. More dilute solutions (about 2 per cent) are sometimes used for treatment of wounds, infected ears, etc.

It must be remembered that concentrated carbolic acid is a violent corrosive poison if taken internally.

Hexylresorcinol, a compound related chemically to phenol and marketed in a mixture called S.T. 37, is an effective disinfectant, provided not too much organic debris is present.

(2) *Cresol and lysol.* Cresol is another product of coal-tar distillation. It has a higher germicidal power than pure phenol and is less poisonous. However, it is not so readily soluble in water. Most commercial disinfectants contain cresol in some form, combined with tar oil, soap, or some other substance. A useful cresol preparation is *Liquor Cresolis Compositus, U. S. P.,* which contains cresol mixed with a soft soap. A 2 per cent or 5 per cent solution of this compound in water makes an excellent disinfectant for feces or sputum, and for general purposes.

Lysol is a mixture of cresol and a linseed oil soap. It is more soluble than pure cresol and makes a milky emulsion in water. Lysol is one of the most valuable disinfectants known; it has many uses in the hospital and in the home. Its action is not interfered with by organic matter. It can be used in 2–5 per cent solutions for disinfection of feces, sputum, and similar materials, for contaminated bed linens, and for the hands of hospital attendants. It is poisonous and is for external use only.

(3) *Formalin.* Formalin is a 40 per cent solution of formaldehyde gas (HCHO) in water. 1 part of this solution with 9 parts of water makes a good disinfectant which is an effective germicide even in the presence of considerable albuminous matter, as in sputum; it is less poisonous than lysol and does not injure most articles. The solution is unstable, however, and hence is reliable only when freshly prepared. Formalin has a hardening as well as a preservative effect upon tissues and is universally used

for the preservation of anatomical and pathological specimens. In the hospital formalin solutions are sometimes used to disinfect rubber gloves or surgical instruments.

(4) *Chlorine and its compounds.* Chlorine as a gas, or as a liquid, or in combination with various other elements, is one of the most effective and most widely applicable germicidal agents we possess. *Chlorine gas* and *liquid chlorine* are used extensively for the sterilization of drinking water, swimming pool water and sewage. About 0.5 part of active chlorine to a million parts of water is the usual amount needed.

The most widely used chlorine compound is *chlorinated lime*, $Ca(ClO)_2$, also called calcium hypochlorite, chloride of lime, or bleaching powder. This substance is made by passing chlorine gas through freshly slaked lime. It is a white powder which has a strong odor of chlorine. The chlorine is not very firmly bound to the compound, therefore the powder decomposes and becomes useless as a disinfectant rather rapidly when exposed to the air. The dry powder may be used, or a solution may be prepared by adding a pound of the powder to two gallons of water. This solution may be used to disinfect feces or sputum, but its action is considerably interfered with by organic matter and an excess of it must be used for such materials. Chlorinated lime was formerly used extensively for the disinfection of drinking water, swimming pools, and sewage, but gaseous and liquid chlorine are now more commonly employed for these purposes. It is one of the cheapest and best chemicals we have for disinfection of such places as dairies, slaughter houses, cellars, outhouses, etc.

Ordinary solutions of calcium hypochlorite or other hypochlorites are too irritating to be applied directly to wounds, because they are so strongly alkaline. But neutral nonirritating solutions have been prepared by special methods. The best known is *Dakin's solution.* The active ingredient in this preparation is *sodium hypochlorite* (NaOCl). As with all chlorine compounds, the germicidal action of Dakin's solution is almost instantaneous and once the solution has reacted with the blood, pus, and germs in a wound it has no further effect. Since Dakin's solution does not irritate the tissues, it may be applied to the wound at frequent intervals or a fresh solution may be made to pass continuously through the wound.

In addition to the hypochlorites, the chlorine compounds called *chloramines* are used in disinfection. Chloramines are organic compounds containing hypochlorites. They decompose to yield free chlorine, just as bleaching powder does, but they are somewhat more stable. They are not irritating to the tissues and therefore can be used in open wounds. The most useful of these compounds are *Chloramine T* and *Dichloramine T*. Chloramine T in 2 per cent solution may be used to irrigate wounds or the dressings may be saturated.with it. Solutions of Dichloramine T in oil are sometimes sprayed upon wounds. Chloramine T, when combined with benzoic acid makes a fairly stable compound which is available in the form of tablets called Halozone Tablets. Fresh Halozone Tablets are useful to campers and travelers who may wish to disinfect a water of doubtful purity. It should be remembered, however, that *boiling* is the best method of disinfecting a small amount of water.

(5) *Lime.* Lime (CaO) is obtained by driving off carbon dioxide (CO_2) from native calcium carbonate ($CaCO_3$). When lime is mixed with about half its weight of water, it becomes calcium hydroxide, $Ca(OH)_2$, or *slaked lime.* If lime is exposed to the air, it will absorb water slowly and become "air-slaked." On further exposure, it reverts to the form of the carbonate. The carbonate has no germicidal power, therefore air-slaked lime cannot be relied upon as a disinfectant. But if fresh lime, which has not been exposed to the air, is slaked by mixing it with water, and then more water is added (to about 4 times the weight of the lime) to make a thick pasty material called *milk of lime*, or still more water is added to make a thinner suspension familiarly known as *white wash*, we have an excellent disinfectant. Freshly prepared milk of lime is especially useful for disinfection of feces.

Disinfectants of more limited use. There are many kinds of disinfectants of more or less limited usefulness employed in medical practice. Among the most important are (6) acids, (7) alcohol, (8) iodine, (9) bichloride of mercury and other mercury compounds, (10) silver nitrate and other silver compounds, (11) salvarsan and other arsenic compounds, (12) hydrogen peroxide and other oxidizing agents, (13) dyes, and (14) essential oils.

(6) *Acids.* All of the mineral acids, like hydrochloric acid, are germicidal, but have a very limited practical use as disinfectants. Fuming nitric acid is sometimes applied to the wound

caused by the bite of a mad dog in an effort to kill the virus of rabies (hydrophobia) that may be there. *Picric acid,* a yellow dye, is commonly used on the human skin after a burn and also to disinfect an area of the uninjured skin. *Boric acid* has a weak antiseptic action and is often used as an eye wash. Hot boric acid solutions are employed in wet packs applied to boils or similar lesions. Vinegar, which contains about 6 per cent *acetic acid* is germicidal. A 1 per cent solution of acetic acid is sometimes applied to the dressings in the treatment of infected wounds.

(7) *Alcohol.* The stock solution of ethyl alcohol (C_2H_5OH), as purchased by laboratories and hospitals, consists usually of about 95 per cent of pure (absolute) alcohol. In this form alcohol is *not* a good disinfectant. But when 95 per cent alcohol is diluted with water to a concentration of 60 to 70 per cent it becomes a useful germicide. *However, its action is slow and uncertain and it is less efficient than many other agents.* Alcohol is most commonly used to disinfect a small area of the skin, and for the hands and arms of surgeons and nurses when they "scrub up" for an operation. Like formalin, alcohol is also employed as a preserving fluid for anatomical specimens.

(8) *Iodine.* Iodine is closely related to chlorine chemically, and it is not surprising to find that it is also germicidal. It is not so powerful a germicide as chlorine, however. It is most commonly employed in the form of a tincture. *Tincture of iodine* is an alcoholic solution. It is made in various strengths; most commonly it contains about 6 per cent of iodine. As a disinfectant for cuts and minor wounds, and for small areas of the healthy skin, tincture of iodine is perhaps the most widely used of all chemical agents. Care must be taken not to apply it in too concentrated a form, because it may burn the tissues. The tincture is most active when freshly made.

(9) *Mercury compounds. Bichloride of mercury* ($HgCl_2$), also called "corrosive sublimate," is one of the most powerful of chemical germicides. Bacteria are quickly killed even by the very dilute solutions (1–1000 or 1–2000) which are commonly employed. Despite this great germ-killing power, it has a number of undesirable properties which distinctly limit its practical value. It is a very poisonous substance if taken internally. Bi-

chloride of mercury solutions should be carefully labeled, and colored with a little methylene blue so that they cannot be mistaken for harmless liquids. It is unreliable as a disinfectant for feces, sputum, or other albuminous materials. In strong solutions it is irritating to the skin. It is corrosive and cannot be used for disinfection of instruments or other metal objects. In the hospital the use of bichloride of mercury is restricted almost entirely to the sterilization of glass and rubber articles, such as catheters and clinical thermometers.

Although mercury is poisonous to the human body, small amounts of the bichloride can be injected into the muscles without harm, and mercury ointments may be applied to the skin or mucous membranes. Mercury is used in these ways as a part of the treatment of syphilis.

Mercurochrome, *metaphen*, and *merthiolate* are recently developed mercury compounds marketed as germicides. Mercurochrome, despite a considerable popularity, is only a moderately strong antiseptic, and not a reliable disinfectant for the skin or mucous membranes. Best of these preparations is merthiolate, which has a greater germicidal power than phenol, may be used as a skin disinfectant, and has been found especially useful as a preservative for vaccines, antisera, and similar materials.

(10) *Silver compounds.* Various compounds of silver are germicidal and several are employed in medical practice. In dilute solution they are not irritating to delicate tissues, therefore they are commonly used in the treatment of gonorrhea and other infections of the genitourinary passages, and for inflammations in the nose, throat and eyes. The best known are argyrol and silver nitrate ($AgNO_3$). It is now the practice everywhere for the doctor, nurse, or midwife who assists at the birth of a baby, to introduce a few drops of a dilute solution (about 2 per cent) of silver nitrate into the eyes of the newborn child in order to prevent gonorrhea and other forms of ophthalmia neonatorum (Credé's method).

(11) *Arsenic compounds.* Arsenic, like mercury, is poisonous to human beings, but small amounts of some of its compounds may be introduced into the body without harm. Arsenical preparations kill spirochetes with especial readiness, and seem to have, in fact, an almost specific action against microbes

of this kind. They are, therefore, of great value in treating diseases in which spirochetes play a part, as in Vincent's angina, bronchiectasis and lung abscess, and similar infections. *Syphilis* is caused by a spirochete *(Treponema pallidum)* and this dread disease is treated by injecting into the body an arsenic compound known as *salvarsan* (arsphenamin or "606"). This famous remedy was first prepared in 1909, after years of experimentation, by the great German scientist, Paul Ehrlich. Neosalvarsan is a modified form of this compound.

(12) *Oxidizing agents. Hydrogen peroxide* (H_2O_2) is a popular but not very reliable disinfectant. It gives up oxygen very readily and in the process will destroy bacteria, provided it is in intimate contact with them. But when applied to a wound or to the mucous membrane of the mouth or elsewhere, it is decomposed very rapidly by reacting with the blood or pus or other organic matter present and its practical effect as a germicide is often slight.

Potassium permanganate is another antiseptic which acts as an oxidizing agent. Its action, like that of hydrogen peroxide, is weakened by the presence of organic matter. It is sometimes employed in treatment of infections of the genitourinary tract.

(13) *Dyes.* A number of the dyestuffs used for coloring fabrics, and for the staining of smears in bacteriology are germicidal. Some of these dyes, such as *acriflavin*, *gentian violet*, *brilliant green*, and *fuchsin*, are used in medical practice. They are most commonly employed for the treatment of infected wounds, and of acute infections in the mouth, urethra or vagina. Their germicidal effect varies a great deal under different circumstances; they are sometimes efficient disinfectants, but in other cases are of little value. They act principally as bacteriostatic agents.

Gentian violet, methylene blue, and some of the other dyes, as a rule kill *Gram-positive* organisms (such as staphylococci and streptococci) more readily than Gram-negative ones (such as colon bacilli and typhoid bacilli). Gram-positive cocci are the principal causes of infected wounds, abscesses and acute inflammations generally, and these dyes are, therefore, especially valuable for treatment of this kind of disease. In order to retard or prevent the growth of certain organisms, and so give others a better chance to develop, a minute amount of brilliant green, gentian violet, or other dye, is sometimes incorporated into artificial culture media.

Sulfanilamide. In 1935 attention was first called to the remarkable power of a synthetic dye called *prontosil* to protect experimental animals against streptococcus infection. Soon it was found that the related chemical compound called *sulfanilamide* (para-aminobenzene-sulphonamide), is also effective, and at the same time is so little toxic that it may be injected in considerable amounts without apparent harm. Naturally these promising findings led to extensive trials of the new chemicals in the treatment of infectious diseases in human beings. So far as can be judged at the present time, the results are most encouraging. The dyes, which are now marketed in tablet form for taking by mouth as well as in liquid form for injection, have been found to be of distinct value in the therapy of the various clinical forms of infection due to hemolytic streptococci, and also in meningitis and gonorrhea, and probably are helpful in still other infectious diseases. Already new derivatives of sulfanilamide have been prepared which are said to be of curative value in pneumonia, one of the most fatal of diseases. Discovery of these dyes may well prove to be the most important step yet made toward the successful chemical treatment of infection.

(14) *Essential oils.* The essences or oils of clove, wintergreen, thyme, peppermint, etc., have a weak germicidal action. They are often included in tooth pastes, cough drops, and similar preparations, but they are seldom, if ever, present in sufficient amount to have any effect. Thymol is of value in treating certain fungous infections.

Germicidal action of soaps. In general, soaps are not to be relied upon as disinfectants. Even the highly advertised so-called germicidal soaps do not in themselves kill bacteria very readily. It is true that soap and water are very important agents in practical disinfection. However, this is *not because the soap solution itself kills bacteria, but because washing and scrubbing removes and destroys many bacteria mechanically.* The more delicate pathogenic organisms, such as those of meningitis, gonorrhea, and syphilis, pneumonia, measles, scarlet fever, and diphtheria, will not survive on the skin after a thorough and careful scrubbing with warm water and any good cleansing soap. But staphylococci, and the germs of typhoid and of dysentery, and many other bacteria, may not be killed by even a prolonged exposure to soap solutions. When the hands are contaminated by the more resistant germs, they cannot be safely disinfected by soap and water alone; after scrubbing, lysol, alcohol, or some other disinfectant must be used. The clinical thermometer is not sterilized by the soap solution on the thermometer tray, but only after it has been first cleansed by the soap, *wiped dry*, and *then* immersed for a sufficient time in bichloride of mercury or other disinfectant.

Specific chemical remedies for germ diseases. Very few germ diseases may be cured by the administration of a particular drug, although in many cases chemical remedies are valuable parts of the treatment. The difficulty is in finding a chemical, to be taken by mouth or injected into the body, which will destroy the germs within the tissues and not severely injure the body itself. Bacterial cells are so nearly identical, so far as the fundamental nature of their protoplasm is concerned, with the cells of the human body, that nearly every chemical which poisons them poisons the body also.

For a few diseases, however, chemical remedies have been found which will destroy the germs within the body without injury to the patient. *Malaria* is cured by *quinine*, which acts specifically against the malarial parasites, the spirochetes of *syphilis* are killed by injections of *salvarsan*, and *leprosy* is thought to be improved by administration of *chaulmoogra oil*. The germs concerned in all of these diseases are quite different from the ordinary bacteria—the malaria organisms are protozoa, the syphilis spirochete is closely related to the protozoa, and the leprosy bacillus belongs to the class of higher bacteria. It seems possible a chemical remedy may some day be discovered for tuberculosis, for the germ here also has a peculiar structure and chemical makeup, but although many preparations have been tried, none have proven successful up to the present time.

The recent discovery of sulfanilamide has brought renewed hope that the successful chemotherapy of bacterial diseases may some day be commonplace. The action of this new remedy is not directly germicidal, but it seems to inhibit the development of the germs in the body and to make them more susceptible to the normal body defenses, especially the phagocytic cells, with the result that the infection is overcome.

REVIEW QUESTIONS—CHAPTER XVIII

1. What are some of the uses of disinfectants?
2. Name the three factors which affect the germicidal action of chemical agents, and explain the influence of each of these factors upon the germicidal power.
3. What disinfectant is taken as the standard for measuring the germicidal power of other disinfectants? Define *phenol coefficient.* If a disinfectant were completely studied, what effects would it be likely to have at different concentrations?
4. Name the five disinfectants which are most reliable for general use.
5. Describe the nature, and discuss the practical use and value of (1) carbolic acid, (2) cresol and lysol, and (3) formalin.
6. What practical uses have gaseous and liquid chlorine? Describe the nature, and discuss the practical use and value of chlorinated lime, Dakin's solution, and the chloramines.
7. Explain how lime may be used as a disinfectant. Define slaked lime, milk of lime.
8. Name nine kinds of chemicals having a more or less limited use as disinfectants.
9. Explain the practical use and value of (1) acids, (2) alcohol, and (3) iodine.
10. How is bichloride of mercury used as a disinfectant? What undesirable properties limit its practical value? Name another mercury compound commonly used.
11. Name two compounds of silver which are widely used. Describe one important use of silver nitrate.
12. What kinds of microbes are especially affected by arsenic compounds? What is salvarsan?
13. Discuss the nature, practical use and value of hydrogen peroxide and potassium permanganate.
14. Name four kinds of dyes which have a germicidal action. What kinds of bacteria are most readily killed by gentian violet and related dyes?
15. Discuss the value and importance of sulfanilamide and related compounds.
16. What value have essential oils as germicides?
17. Discuss the value of soaps as disinfectants. What kinds of disease germs might be killed by simple scrubbing with soap and water? Give examples in which exposure to soap solutions is not of itself sufficient for disinfection.
18. Name three germ diseases successfully treated by chemicals which specifically destroy the germs within the body. Name the chemical agent in each case.

CHAPTER XIX

SOURCES OF INFECTION. THE SPREAD OF DISEASE BY PERSONAL CONTACT

IT IS ONLY in comparatively recent years that we have come to understand how we "catch" disease, and how disease germs are transferred from one individual to another. Widespread epidemics of cholera, typhoid fever, smallpox, and other serious diseases were familiar enough a hundred years ago, but their origin and manner of spread were not understood. Most of the theories advanced to explain these epidemics were based upon the belief that some evil influence was exerted by the surroundings in which people lived. Epidemics were said to be caused by some unusual configuration of the stars, by earthquake, or volcanic eruption, or by a peculiarity of the weather. The causes of disease were thought to reside in something *external* to the bodies of men.

Even after the work of the pioneer bacteriologists, during the last quarter of the nineteenth century, had demonstrated that most of our common diseases are caused by microscopic germs, there was still a great deal of misunderstanding as to the way these germs reach our bodies and pass from person to person. It was commonly believed, for example, that disease was spread through poisoned air. Indeed, there are many persons even today who fear night air and "sewer gas."

When it was shown, about fifty years ago, that cholera and other intestinal diseases may follow the drinking of water polluted with waste matter from the human intestines, the attention of all sanitarians and medical men was attracted to *filth* in general as a source of disease. It became a very widespread notion that filth of any kind was the most important cause of illness. The efforts of all early public health officers were directed toward cleaning up the environment. Much good was accomplished by this work, for at that time people lived in a much less cleanly manner than they do now. A marked reduction in cases of typhoid fever, and other intestinal diseases, followed wherever general sanitary conditions were improved. But merely cleaning up the rubbish and filth did not prevent the spread of measles, or influenza, or many other communicable diseases. We now understand the reason for this. *The germs of these diseases do not live in filth.* The important fact brought out by modern studies is that *not a single one of the germs which cause communicable diseases has any natural existence outside of the bodies of men or animals. On the contrary, nearly all disease germs die very quickly when cast off from the body.*

It is true that the germs of typhoid fever, cholera, and dysentery may exist in contaminated water or food for a few days, and the germs of tuberculosis, scarlet fever, and a few other diseases may live in milk, but these are exceptions to the general rule. And whenever disease does arise from the use of contaminated food or water, the germs have always been introduced *a short time before* from the discharges of some person or animal.

The true sources of infection. The important point to remember is that most of the disease germs are so closely adapted to life in the bodies of living men or animals, that they can exist only for brief periods of time on any external object. They do not travel through the air. They do not remain alive and virulent for long periods on clothing, baggage, furniture, and similar articles. On the contrary, *germs remain closely associated with infected persons.* Communicable diseases are spread most commonly by *contact with persons*, not things.

The real source of infection lies in the secretions and excretions from the persons or animals harboring the germs. The feces, urine, sputum, nasal discharge, or saliva contain the organisms, and these are the truly dangerous things. It is through actual contact with these body discharges that germs pass from one individual to another. External objects used by infected persons act only incidentally as bearers of pathogenic organisms when freshly contaminated with germ-laden excretions.

Modern methods of preventing the spread of communicable diseases are based upon these conceptions and upon an intimate knowledge of the germs concerned. A pure water and milk supply, adequate disposal of sewage, and, in general, a clean environment are recognized as essential, but the greatest emphasis is now placed upon the discovery, isolation, and treatment of the persons who are carrying germs, and upon the teaching of personal hygiene.

Persons who carry germs. Individuals who harbor germs of specific diseases, eliminate them from their bodies, and therefore act as sources of infection for others, include: (1) *persons who have a typical case of a disease*, (2) *persons who have a mild, atypical, unrecognized case*, and (3) *persons who harbor germs without having any symptoms of disease*—that is, the so-called *carriers.*

Typical cases. Persons who are suffering from a typical case of a communicable disease are carrying, of course, the germs of that particular disease, and obviously they are a source of infection to other individuals who come in contact with them. However, the typical case of any of the common diseases is usually promptly recognized and *isolated*, so that the infection is not likely to spread to any great extent.

Unrecognized cases. Persons whose illness is so mild or so unusual that its true nature is not recognized are much more likely to be responsible for the occurrence of new cases. Mild and atypical cases of many of the common communicable diseases occur frequently. In epidemics of dysentery, scarlet fever, poliomyelitis, and other communicable diseases, there are numerous individuals infected with virulent organisms whose symptoms, nevertheless, are so slight, or so unusual, that a proper diagnosis is never made, and their cases never come to the attention of physicians or public health authorities. Contact with such persons accounts for many of the typical cases which develop in more susceptible individuals.

Carriers. The healthy carrier constitutes a still more important focus from which infection may spread. In many of the common infections—for example, scarlet fever and typhoid fever—there is a marked tendency for the causative organisms to persist in the body of the infected individual for some time after all the symptoms of the disease have disappeared. Many cases of scarlet fever may be traced to contact with such *convalescent carriers* who recovered from their own attack of the disease more than a month before. Some investigations have indicated that about 2 per cent of those who recover from typhoid fever still excrete typhoid bacilli occasionally in the feces or urine for many months after they are entirely well, and in some instances they remain carriers for the rest of their lives.

Another class of carriers includes those persons who harbor virulent germs but have not themselves developed the illness at any time. Usually such a healthy carrier is totally ignorant of the fact that he is a source of infection for others. He mingles freely with his associates and may be the innocent cause of many cases of disease. He is himself immune.

Importance of carriers as sources of infection. Obviously the existence of numerous healthy carriers of disease germs greatly increases the opportunities for the spread of infection. The discovery of carriers, and their control, constitutes the most important and difficult problem in connection with the prevention of diphtheria, scarlet fever, typhoid fever, epidemic meningitis, and other communicable diseases. New cases originate most commonly from contact with carriers, rather than cases, especially in times of epidemic when many individuals become temporary carriers. Studies made in army camps during epidemics of meningitis, for example, disclosed that among the population of the camp there were about ten times as many carriers of the meningococci as there were cases of the disease.

Exit of germs from the body of infected persons or carriers. Different kinds of germs have adapted themselves to different pathways of exit from the body. This depends in large part upon the region of the body principally affected. Thus, the germs of intestinal diseases will be contained in the feces or urine, whereas the organisms causing infections of the respiratory organs will be found in the sputum, saliva and nasal secretions.

The body discharges or secretions by which the principal disease germs are eliminated are as follows:

Feces. Organisms of typhoid fever, paratyphoid fever, dysentery, cholera, diarrhea, glanders, tuberculosis (especially in cattle), anthrax, actinomycosis (in cattle), and possibly poliomyelitis and smallpox. Hookworms and other animal parasites are also found in the feces.

Urine. Organisms of typhoid fever, paratyphoid fever, undulant fever (brucellosis), tuberculosis (of kidneys), possibly smallpox.

Sputum. Organisms of tuberculosis, pneumonia, whooping cough, influenza, pneumonic anthrax, pneumonic plague.

Saliva. Virus of rabies (in mad dog). Also all organisms found in nose and throat discharges, and possibly germs of intestinal diseases.

Discharges from nose and throat. Organisms of all diseases of the respiratory organs and also some diseases not primarily involving the respiratory tract. Most important are measles, mumps, chickenpox, smallpox, whooping cough, influenza, pneumonia, pneumonic plague, diphtheria, scarlet fever, septic sore throat, epidemic meningitis, poliomyelitis, epidemic encephalitis, tuberculosis, syphilis, glanders, actinomycosis.

Secretions from eyes. Organisms of conjunctivitis, and of trachoma.

Pus from discharging ears, or other local lesions. Organisms of scarlet fever, septic sore throat, furunculosis—pathogenic streptococci, staphylococci, and other organisms.

Secretions of lesions of skin and mucous membranes. Virus of smallpox, organisms of leprosy, glanders, actinomycosis, anthrax, favus, gonorrhea, syphilis, chancroid.

Blood (germs carried away by biting insects). Malaria, dengue, yellow fever (mosquitoes). Plague (fleas). Rocky Mountain spotted fever (ticks). Tularemia (flies, ticks). Typhus (lice, fleas).

Principal methods of germ transfer. The most common way in which communicable diseases are spread is through (1) *personal contact* with infected persons or carriers. This may be (*a*) an *actual bodily contact*, or (*b*) an *indirect contact through contaminated hands*, or (*c*) *through the common use of articles soiled by discharges*, or (*d*) *by droplet infection.* Other important methods of germ transfer are (2) *infection through contaminated water*, (3) *infection through contaminated milk and other foods*, and (4) *insect-borne infection.* Of lesser importance are (5) *prenatal or congenital infection*, and (6) *soil-borne infection.*

The last mentioned modes of infection may be dismissed with a few words. The remainder will then be considered more extensively.

CONGENITAL INFECTION

By congenital infection is meant infection transmitted from the mother to the fetus before birth so that the baby is born with the disease. Syphilis is often transmitted in this way, and rarely, other diseases. This is not really inherited disease—that is, the causative organisms are not present in the human germ cells (which contain all we directly inherit),—but rather it is infection acquired during uterine life from the mother, probably by passage of the organisms through the placenta.

SOIL-BORNE INFECTIONS

There is little reason to fear the dust and dirt as sources of infection, because we know that the germs of most communicable diseases do not long survive there. But there are a few instances in which the soil may be regarded as the origin of infectious disease. The germs of tetanus and gas gangrene are deposited on the earth in the intestinal discharges of men and animals and their spores survive for long periods in the soil. When dirt containing these spores is introduced accidentally into a wound, tetanus or gas gangrene may result. In a similar manner anthrax in animals is a soil-borne infection. When germ-laden excreta are deposited upon the soil, and drainage from this contaminated ground pollutes water supplies, causing typhoid fever, the soil might be regarded as a source of infection.

INFECTION THROUGH PERSONAL CONTACT

(*a*) **Actual bodily contact.** Obviously a person may become *inoculated* with germs by coming into immediate contact with lesions in some other person or animal. Except for those diseases transmitted solely by the bite of insects, any of the communicable diseases might conceivably be contracted in this way. This very direct transfer of germs is the rule, however, in only a few diseases. The most important examples are the venereal diseases *gonorrhea* and *syphilis*. The organisms of these infections are so delicate and so closely adapted to the living tissues that they have practically no existence outside of the body. Therefore, these diseases are very rarely contracted except by actual bodily contact with lesions containing the organisms.

The great majority of other infections pass from person to person less directly, and by some one of the methods of indirect contact described below.

(*b*) **Infection through contaminated hands.** First importance must be given to contaminated hands as the means by which disease germs are carried about. A vivid description of the part dirty hands may play in the spread of infection is given by Sedgwick in his report of an outbreak of typhoid fever in 1892, quoted by Chapin.*

> "The families are of that grade in which food always stands upon the table; meals are irregular except for those who must obey the factory bell. The children play awhile, then visit the privies, and with unwashed hands finger the food upon the table. Then they eat awhile and return to play. Or, changing the order of things, they play in the dirt and eat and run to the privy, then eat, play, and eat again, and this in various houses and various privies. For them, so long as they are friendly, all things are common—dirt, dinners and privies; and, to illustrate exactly how secondary infection may go on, I may describe in detail one case which I personally witnessed. A whole family (of six or more) was in one room. Four of them had the 'fever.' Two of these were children in the prodromal stage. A table stood by the window covered with food, prominent among which was a big piece of cake. . . . By and by one of the children having the fever withdrew to the privy, probably suffering with diarrhea, but soon returning, slouched over to the food, drove away some of the flies, and fingered the cake listlessly, finally breaking off a piece, but not eating it. Stirred by this example, another child slid from his seat in a half stupid way, moved to the table, and, taking the same cake in both hands, bit off a piece and swallowed it. The first boy had not washed his hands, and if the second boy suffered from secondary infection I could not wonder at it."

Such ignorance and squalor are not found everywhere, of course, and such conditions are less common now than in 1892. Nevertheless, it must be true that the hands of the best of us are seldom really clean. Most individuals frequently bring their fingers to the nose or mouth. Then they deposit saliva and nasal discharges on the objects they handle. This must result in a continual swapping of bacteria with our associates, who finger the same objects, and who are likewise busily engaged in distributing saliva. The hand-to-mouth, hand-to-nose transfer of germs is probably much more common than is generally realized, and in many instances no more mysterious method need be imagined to account for the spread of communicable diseases.

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* CHAPIN, C. V. Sources and Modes of Infection, John Wiley & Son, 2nd Ed., 1916, p. 169.

(*c*) **Infection through articles freshly soiled with discharges.** Next to fingers themselves as bearers of germs come inanimate objects, such as handkerchiefs, drinking cups, and the like, which have been *freshly* contaminated with secretions or excretions from an infected person or carrier. Such an object is called a *fomite.* Those articles in immediate contact with the patient or carrier and used by him, such as bed clothing, bed linen, tableware, handkerchiefs, towels, toilet articles, emesis basins, and other things used in the sick room, and the candy and toys of children, are most likely to be contaminated with pathogenic bacteria. We know that most of these germs can remain alive on such objects only for a short period of time, but it is easy to see how the common towel and drinking cup in public places, and the innumerable small objects used in common by members of the same household, might occasionally serve to transfer virulent organisms from one person to another.

Among children, especially, freshly contaminated articles probably are important means of spreading germs. Children put all manner of things instinctively into their mouths, and they do not stop to inquire who was mouthing the thing last. In this way children who play together are continually swapping saliva, and a cold or similar infection in one child is quickly passed to the other children. Youngsters naturally have no regard for personal cleanliness; this is something that has to be taught. Very young children do not seem to be able to truly appreciate an object until they have gotten at least a corner of it into their mouths. The habit of carrying the fingers to the mouth and nose, and of putting pins, pencils, and all kinds of other small objects into the mouth, begins very early, and the practice of a stricter personal hygiene, which we know to be essential to prevent infection, begins, if at all, rather late in the lives of most individuals. This largely explains why communicable diseases transmitted by contact with nose and mouth secretions still occur so frequently despite an abundance of knowledge about them.

(*d*) **Droplet infection.** When a person is infected by inhaling droplets of germ-laden moisture expelled from the mouth or nose of another person, the method of germ transfer is referred to as *droplet infection.* This is another very common means of spreading germs. A cough or a sneeze sends a dense spray of moisture into the air, every droplet of which may be loaded with virulent organisms dislodged from the inflamed lungs, bronchi, throat, mouth or nose. A person standing a few feet away can scarcely avoid taking some of these droplets into his own nose or mouth with the breath. In this way the germs of diphtheria, influenza, measles, and all the other organisms which may be present in saliva or nasal discharges, are very readily passed from person to person, especially when individuals are brought into close contact in crowded living quarters, street cars, schools, and the like.

The wearing of a gauze mask over the nose and mouth by hospital attendants, while caring for a patient with influenza or other respiratory disease, is commonly practiced as a precaution against droplet infection. Masks are worn also in the children's wards and nursery, primarily for the protection of the patients. The surgeons and nurses in the operating room are masked to prevent droplets from contaminating the operative field.

Importance of contact infection. Almost everyone has learned through actual experience that disease may be contracted by personal contact with the sick. From what has been said about carriers, the student will understand that an infection may also be acquired by contact with a healthy person. It is further important to realize that it is by personal contact (through contaminated hands, freshly soiled objects, and droplet infection) that the great majority of infections of all kinds (except insect-borne diseases) are transmitted. Epidemics of typhoid fever and other intestinal diseases, caused by contaminated milk or water, are so spectacular that we tend to forget how important a rôle contact infection plays in the spread of the very same diseases. As a matter of fact, in intestinal infections, as well as in measles, influenza, meningitis, and other common diseases, the methods of germ transfer discussed above play the principal part.

Numerous instances might be given of epidemics in which the causative organisms were transmitted solely through personal contact. Naturally such outbreaks are likely to be most frequent in groups of persons crowded together in schools, institutions, or army camps, and so it is not surprising to find that diseases like measles, diphtheria, and epidemic meningitis are most common among school children and soldiers in camp. In a *hospital* there is a community of persons brought into unusually close contact, and the danger of the spread of infection by personal contact from patient to nurse, or from nurse to patient, is very real. In the days before aseptic methods were used, hospital attendants often innocently carried infection from patient to patient, usually by way of contaminated hands, and frequently themselves contracted serious illnesses. Now contact infection within the hospital is prevented by cleanliness, and the conscientious practice of aseptic technique. The practical means of avoiding the spread of infection in the sickroom and the operating room are discussed in the following chapter.

Personal hygiene versus public sanitation in the control of communicable diseases. It will be clear from the foregoing discussion that the maintenance of our individual health, and incidentally the public health, depends in large part upon how well we practice personal cleanliness, and personal hygiene in general. The measures taken by public authorities to purify the water supply, safeguard the purity of milk and other foods, dispose of sewage and other wastes, destroy insects, improve housing, and otherwise to promote sanitation are also important, indeed they are indispensable, but they have a more limited effect in directly preventing communicable diseases. Unfortunately it is not true, as many laymen are prone to believe, that if only sanitary facilities could be perfected everywhere most of our ills would vanish. No amount of public sanitation has any direct effect upon the prevalence of smallpox, or influenza, for example, or upon the great host of other diseases commonly spread by personal contact. It took the human family a long time to learn the saving virtue of personal cleanliness, but now every nurse, doctor, and health worker seeks to promote by every possible means the practice of personal hygiene, physical and mental, in all its aspects, as the most direct way of preventing disease.

REVIEW QUESTIONS—CHAPTER XIX

1. Contrast older ideas with present knowledge concerning the sources of infection and the spread of disease.
2. Why is it that a pure water and milk supply, and good sanitary conditions are not sufficient in themselves to prevent the spread of all communicable diseases?
3. State concisely what are the true sources of infection.

4. What three classes of persons carry germs? Explain the danger of the unrecognized case.
5. Define *carriers* and explain their importance as sources of infection.
6. Name some of the common diseases in which the germs are eliminated in the (*a*) feces, (*b*) urine, (*c*) sputum, (*d*) nose and throat discharges. What germs may be present in the (*e*) saliva, (*f*) skin or mucous membrane lesions, (*g*) pus, (*h*) blood.
7. Name six common methods of germ transfer, and indicate which are the more important.
8. What is meant by congenital infection? Name one disease often transmitted in this way.
9. What importance has the soil as a source of infection?
10. What are the four forms of personal contact by which germs may be spread?
11. Give examples of diseases transmitted by actual bodily contact.
12. Discuss the importance of contaminated hands in the spread of infection.
13. Under what circumstances may inanimate objects be a source of infection? Define *fomite.*
14. Define *droplet infection* and explain its rôle in the spread of disease.
15. Discuss the importance of personal contact as a method of germ transfer.
16. Compare personal hygiene and public sanitation as factors in controlling communicable diseases.

CHAPTER XX

PREVENTION OF CONTACT INFECTION. PRACTICAL ASEPTIC TECHNIQUE.

WHILE THE practice of personal cleanliness to minimize chances of infection should be an everyday matter, the need for careful habits becomes acute in those who are called upon to care for a sick person, particularly if the patient suffers from an easily communicated infectious disease. The responsibility of avoiding infection is again especially great upon the doctors and nurses who take part in surgical operations. The practical methods of sterilization and disinfection and the general techniques which may be used in the sickroom and in the operating room are outlined here.

IN THE SICKROOM

Not every disease is caused principally by germs, but there are very few severe illnesses of any kind which are not complicated, at least, by an infection of some part of the body. And even though the patient does not suffer from any form of germ disease, many details of his care require the application of microbe-destroying methods.

Obviously, the use of these methods becomes especially important when a case of a disease caused primarily by germs must be cared for, because *nearly all germ diseases are more or less readily communicated from one person to another.* The doctor and nurse who are called upon to attend such a case must assume a very grave responsibility. Their task is not merely to aid in the recovery of the patient, but also to *prevent the spread of the disease* to themselves or to others.

This means that they must carry out a very rigorous *aseptic technique;* that is, they must practice those *methods by which germs are excluded and infection is prevented.* They must try to avoid infection of their own persons, and they must also avoid carrying the disease to others. They must see to it (the nurse especially has this responsibility) that, as far as is humanly possible, all the dangerous germs cast off from the body of the patient are destroyed before they have an opportunity to infect another susceptible person. They must also endeavor to prevent reinfection of the patient with the germs already injuring him, and try to avoid the entrance of other harmful microbes into his weakened body.

Isolation of cases of communicable germ disease. The first step necessary to prevent the spread of germ diseases is the *isolation* of the patient. In most hospitals patients who have any of the common germ diseases are isolated. If the case is one of the more easily transmitted diseases, such as measles, whooping cough or other infections of children, or smallpox, influenza, diphtheria, meningitis, or typhoid fever, the isolation technique must be very strict indeed. The isolated patient is given a room to himself, or in some cases his bed is merely separated from other beds by a glass partition or a screen. It is not necessary that the patient be walled in, and even the screen may be dispensed with, and the bed marked only with a chalk line on the floor, because germs do not fly through the air. In practice, the isolated patient is usually segregated in a separate room or cubicle of glass, when space permits, because it is more convenient to carry out isolation technique under those circumstances, and the presence of some kind of physical barrier about the patient serves as a reminder to the nurse and others that the patient is a source of danger. The important point is that the isolated patient is cut off from direct contact with all other persons, except those who must necessarily attend him. Isolation means that the patient and his immediate surroundings become like the bacteriologist's culture tube containing powerful pathogenic bacteria. *The organisms must not be permitted to escape so as to cause harm elsewhere.*

General precautions in the care of the isolated patient. The technique used in the care of the isolated patient is essentially nothing more than the application of common sense to the problem of preventing the spread of the disease and has its basis in definite knowledge of the ways of microbes. The actual methods of disinfection employed and the details of the isolation technique naturally differ in different hospitals because more than one agent or method may be used to achieve the same result. And any of the methods must be modified, of course, to meet the actual conditions in a private home.

Cleanliness. Whether the patient is in the hospital or at home, cleanliness in the sickroom is a primary consideration. Truly clean things do not carry germs, and cleanliness really forms the very basis of disease prevention.

The sickroom should contain no unnecessary furnishings, so that everything in it can be truly cleaned. Dust must be removed, but it should not be stirred up into the air. Sweeping is best done with soft brushes and after wet sawdust has been sprinkled upon the floor. The dust rag should not be dry, but should have a little oil on it. There is little reason to fear room dust as a carrier of disease germs. It is possible, however, that some pathogenic organisms, such as the tuberculosis germs or the spores of pathogenic molds or higher bacteria, might exist in the dust of a dirty room in which a patient, who has been excreting these germs, has lived for a while. At any rate, dust cannot be tolerated in the sickroom. *The floor, the woodwork, and the bed frames should be scrubbed frequently and vigorously with hot water and soap.* In addition, a disinfectant, such as cresol, lysol, chlorinated lime solution, or carbolic acid, may be applied to all surfaces. The most important cleansing agents, however, are hot soap suds and "elbow grease." Sprinkling around a little disinfectant will have no effect, but mechanical destruction of microbes by frequent scrubbing is very much worth while. This kind of cleaning does two things: it removes many bacteria bodily, and also it removes the organic matter in which they may exist.

The person of the patient himself must be kept as clean as possible. Prompt disposal of all soiled bedding, dressings, and discharges of any sort will go a long way toward preventing reinfection of the patient and spread of the disease. In cases of intestinal disease and in other cases where necessary, the *patient's hands* should be disinfected before eating. They may be scrubbed with soap and water, then held in a basin of 50 per cent alcohol for two minutes, and dried on a sterile towel.

Food and drink for the patient must be protected from contamination. The nurse who is caring for a case of typhoid fever or other intestinal disease should have nothing to do with the preparation of food for the patient or for his family. In the hospital, and in the home as well, cleanliness in the kitchen is of prime importance. Flies and vermin of all kinds must be kept out of food. Milk, vegetables, and other perishable foods must be protected from dirt and dust, handled with clean hands, and kept cold in a clean refrigerator.

Conduct of the doctor and nurse in the sickroom. Obviously the doctor and nurse in attendance upon an isolated patient must conduct themselves with care. Immediately upon entering the room, or before approaching the bed, the nurse and doctor put on long *gowns* which cover them from the neck to the feet. The gowns are always handled in such a manner that the outside surface (possibly contaminated) is not touched by the clean hands. The nurse may wear a special cap to cover her hair. If the case is one of influenza, pneumonia, or any one of the many other diseases of the respiratory organs in which the germs are present in the nose and mouth secretions, and are commonly transferred to others by coughing or sneezing, the nurse and doctor may wear a *gauze mask* over their faces. Sometimes when it is necessary to touch the patient or to handle materials which might grossly contaminate the hands, *rubber gloves* are worn.

Visiting an isolated patient is usually forbidden. If visitors are permitted, they, too, must wear gowns. When ready to leave the patient, the doctor or nurse first remove their rubber gloves. These are usually disinfected by soaking them in a bichloride of mercury solution, or lysol solution, or they may be boiled. The hands are next disinfected, whether gloves have been worn or not. Then the gown is taken off, with proper precautions not to touch the contaminated outer surface. The soiled gown is sterilized by soaking it for several hours in a solution of lysol or other disinfectant, by boiling, or by autoclaving. The gauze masks must be changed frequently, and after use should be burned, boiled, or soaked in a disinfectant.

Disinfection of the hands after contact with the patient. By far the most important of the steps in the technique just outlined is the disinfection of the hands. This step is indispensable, whether or not gloves have been worn, and whether or not the hands have been knowingly contaminated. The careful nurse will take pains not to contaminate her hands unnecessarily, but it will be practically impossible to avoid getting some germs on the fingers from the patient's body, or from some articles soiled by his excretions. And the hands of the nurse and doctor are more likely than any other thing to be the means by which the germs are carried away and perhaps transferred to some one else. *The commonest method of germ transfer is by way of contaminated hands.* It is, therefore, of the utmost importance that the nurse and doctor conscientiously disinfect their hands before they leave the patient's bedside, and under no circumstances must they approach another patient until this has been done.

Methods and rules of hand disinfection after contact with an isolated patient vary in different hospitals. The safest method is to give the hands and forearms a vigorous and prolonged scrubbing with soap and water. Two minutes of vigorous scrubbing with a stiff brush, warm water (preferably running water), and soft soap, giving special attention to a thorough cleaning about the nails, is sufficient if *conscientiously carried out.* The scrubbing should not be so vigorous as to break the skin. There should be no rings on the hands. After the washing, the hands may be placed for two or three minutes in lysol, alcohol or other disinfectant. Where running water is not available it is the usual practice to soak the hands *first* in the disinfectant, rubbing them over with a brush kept in the solution, then to wash them in soapy water. Sometimes the use of a disinfectant can be safely omitted, because the more delicate disease germs will be destroyed and removed by the scrubbing alone, but if the case is one of typhoid fever or other intestinal disease, or one in which staphylococci or spore-bearing bacilli are abundant, it is far better to use a good chemical germicide. It must always be remembered that the careful washing cannot be omitted under any circumstances, and also that the mere dipping of the hands into a strong solution of a disinfectant is of no value. The disinfectant solution should be diluted to the proper concentration and the hands should be covered with it for at least two minutes.

In many hospitals, cases of measles, diphtheria, and other highly contagious diseases are successfully cared for in the same ward, and often in adjacent beds, and the nurses pass from bed to bed without carrying infection from one patient to another, when hand disinfection after every contact is carried out carefully.

Concurrent disinfection of articles contaminated by the patient. Aside from hand disinfection, the most important feature of the isolation technique is the thorough disinfection of all articles used by the patient and all discharges from his body *continuously* throughout the illness, and so long after recovery as the patient is still carrying the disease germs. This is spoken of as *concurrent disinfection.*

Eating utensils. The isolated patient should have his own set of dishes and tableware and they should be kept apart for his exclusive use. They must be disinfected after use by boiling them in soapy water, and they should be rinsed finally in very hot water. The tray on which the food was served may be boiled or disinfected with a chemical solution. Remnants of the patient's food should be burned or thrown into a basin of disinfectant.

Bath water. The bath water should be disinfected before it is poured into the drain. An excess of chlorinated lime may be added and the mixture allowed to stand for an hour.

Bed clothing, bed linen, and other articles used by the patient must be disinfected as soon as possible after they become soiled. Every article that has been in contact with the isolated patient is considered contaminated and must be disinfected before it can be used for another purpose. The bed clothing, sheets, pillow cases, towels, napkins, etc., are gathered up by the nurse, wearing a gown, and taken directly to a waiting pan of water, which is then heated to boiling, or to a steam sterilizer, or they are put to soak in a dilute solution of carbolic acid, lysol, or other disinfectant. Only after they have thus been disinfected, are these articles sent to the laundry. *Rubber articles,* such as hot water bottles, ice-caps, air-cushions, and sheets are usually soaked in bichloride of mercury or other disinfectant solution, then rinsed in clean water. Rubber catheters are boiled after use. *Enameled basins,* pitchers, and pans are thoroughly cleaned in boiling soapy water, and may be autoclaved or allowed to stand in a disinfectant solution. Mops used to assist in cleaning bedpans and urinals are kept constantly in a pail of disinfectant. *Toilet articles* used by the patient are disinfected by boiling, if possible, or by immersion in a solution of a chemical germicide.

Clinical thermometers. The isolated patient has a separate clinical thermometer for his own use. But in the wards one thermometer may serve for several patients. In any case, the thermometer must be sterile when placed in the patient's mouth and after removal it must be sterilized before it is used again. In order to facilitate the handling of clinical thermometers, a "thermometer tray" is prepared in the hospital. On the tray there are glasses or tubes containing (1) soap solution, (2) clean water, and (3) a disinfectant, such as carbolic acid, alcohol, or bichloride of mercury. A 1–2000 solution of bichloride of mercury is probably the best disinfectant for this purpose. On the tray, also, is a receptacle for clean cotton and for soiled cotton. The thermometer is kept immersed in the disinfectant solution, and just before placing it in the mouth (or rectum, for rectal temperatures) it is wiped off with a bit of cotton wet with the clean (not necessarily sterile) water. After the thermometer has been removed from the mouth or rectum, and the temperature recorded, it is carefully and thoroughly wiped with a piece of cotton wet with the soap solution in order to remove all adherent mucus. *This step is absolutely essential, because the germicidal action of the disinfectant is interfered with unless the thermometer is properly wiped.* But it must be remembered, also, that *the thermometer is not disinfected merely by wiping with soapy water, nor even by allowing it to stand in the soap solution. It must be placed, after wiping, into the disinfectant and must be permitted to remain there long enough to sterilize it before it is used for another patient.* Three minutes in 1–2000 bichloride of mercury solution is sufficient.

Concurrent disinfection of discharges from the patient. All of the things in contact with the isolated patient which have been mentioned above—the bed linen, dishes, and so forth—must be disinfected for just one reason, namely, *because they are liable to be contaminated with some of the discharges from the mouth, nose, bladder, bowels, wounds or open sores, of the patient. These excreta—nasal discharge, sputum, urine, feces, pus—are the truly dangerous things.* Some one or more of them (according to the disease) contain the germs which are causing the illness, usually in great numbers, and at the very height of their disease-producing power. The aim of concurrent disinfection is to destroy these germs at the very bedside, before they can carry disease to other persons.

Discharges from the nose should be received on gauze or cloths, which may be collected in a paper bag, and the bag and contents burned. Handkerchiefs may be soaked in solutions of lysol, cresol, carbolic acid, or chlorinated lime, or thoroughly boiled.

Sputum must be collected and disinfected with the greatest care. Probably the best method is to collect the sputum in paper cups which may be placed in paper bags, and then burned. Sometimes an enameled cup with a cover which can be raised with the thumb is used, or a wide-mouthed bottle. The cup or bottle should contain about 10 c.c. of 5 per cent carbolic acid solution or a 10 per cent formalin solution. This amount of chemical cannot be relied upon to completely disinfect the sputum, however, and the vessel and its contents must be disinfected by autoclaving, or by soaking for several hours in an excess of a disinfectant. The sputum of patients with advanced tuberculosis of the lungs is especially dangerous, because there is likely to be a great deal of it, and the patient, and often the nurse and doctor as well, become indifferent to its danger. Tuberculous sputum should never be carelessly handled, and every drop of it should be conscientiously disinfected.

Feces and urine from patients with typhoid fever and other intestinal diseases must always be disinfected. Great thoroughness and care are required. The disinfection of *feces* is best carried out in the bedpan. Lysol, formalin, milk of lime, or carbolic acid, in an amount at least twice the volume of the feces, should be added and the fecal masses broken up and thoroughly mixed with the solution. The mixture should stand at least two hours before the pan is emptied. The empty pan may be disinfected by pouring in about a quart of disinfectant, allowing it to remain there for an hour or two, or by placing the entire pan in boiling water or in a steam sterilizer. *Urine* may be disinfected by allowing it to stand for a time with an excess of chlorinated lime. *Vomitus* may be disinfected by chemicals, or burning.

Pus from wounds and open sores is best collected on pieces of gauze, which may be dropped into paper bags, then burned.

Terminal disinfection. Disinfection of the bed and room occupied by the isolated patient after his removal (*terminal disinfection*) is not nearly so important as the concurrent disinfection just described, which was practiced while the patient was there. *Persons, not things, are the most important sources of infection,* and when the infected person is removed, most of the danger is gone. If cleanliness has been insisted upon, and concurrent disinfection properly carried out during the illness, the bed and the room can be made ready for use again very easily.

Fumigation of rooms and contents with formaldehyde gas after use by a case of a contagious disease was formerly practiced very widely everywhere. But now it is not often done, for it has been found that fumigation is troublesome, expensive, and often ineffective, and, furthermore, it is unnecessary.

The simplest and best procedure is give the room and contents a thorough scrubbing with hot soapsuds, followed by wiping of the bed, furniture, door-knobs, and other surfaces with a dilute disinfectant. In a private home re-painting and re-papering the room may seem desirable, but it is not at all necessary. The room should be thoroughly *sunned and aired.* The bed, blankets, and the mattress should be exposed directly to the sun out of doors. If the mattress is very badly soiled it may be necessary to take it apart, wash and sterilize the filling and put it together again, or the entire mattress may be sterilized in a large autoclave.

IN THE OPERATING ROOM AND DRESSING ROOM

Aseptic technique in modern surgery and obstetrics. The most conspicuous feature of modern surgical practice is the scrupulous care taken to avoid infection of the operative wound. Every surgeon endeavors to carry out in minutest detail a strict aseptic technique. If he operates in a sterile field, he tries to keep it sterile. If he operates in a field already infected, as he is often obliged to, he tries to stop the infection and to prevent it from spreading beyond the wound. Similarly, the obstetrician takes the greatest care to avoid infection of the uterus at childbirth.

The vital importance of avoiding infection at operation and at childbirth can scarcely be overestimated. Many operations, especially those in which the head or the abdomen is opened, are certain to lead to death if pathogenic organisms enter the operative wound. Hence, the perfection of the aseptic technique at operation is as important for the welfare of the patient as the skill of the surgeon.

Nurses must understand this aseptic technique very thoroughly and learn to practice it conscientiously, because they are directly responsible for it and everything depends upon how intelligently and faithfully they meet this responsibility.

Surgery before the use of germ-excluding methods. We of a more fortunate and enlightened generation can scarcely appreciate the horrors of the older time, when, to the hazards of an operation itself, no matter how trivial, was added grave danger from the infection that inevitably followed. In those days, before the introduction of antiseptic methods by Lister (in 1867), practically every wound, whether accidental or deliberately made by a surgeon, became a running sore, discharging quantities of pus. This was regarded by the best surgeons of the day as inevitable and even necessary for the healing of the wound. The ill-smelling "matter" from wounds was so highly regarded that it was referred to as "laudable" pus.

It seems a wonder that any patients recovered from an operation or survived an extensive accidental wound. Some of the more fortunate did recover, however, but, as a rule, only after weeks of pain and fever. Their wounds improved slowly, requiring three or four weeks, and often much longer, for complete healing. Many other patients did not fare so well and the end was more commonly tragic than happy. The majority of wounds of all sorts became infected with powerful disease-producing germs, and the patients developed *erysipelas* (a streptococcus infection of the surrounding skin), or *tetanus* (lockjaw), or "blood poisoning" (a generalized infection of the entire body). Many wounds were invaded by the worst disease of all, a horrible gangrene which caused the flesh to blacken, putrefy, and rot away. The latter infection was so common in hospitals seventy or eighty years ago, especially in the overcrowded, ill-ventilated, unsanitary surgical wards of charity hospitals, that it was known in every country as *hospital gangrene.* The gangrene became epidemic and spread from patient to patient. It is said that the wards reeked with the nauseating smell of rotting flesh and frequently resounded with the piteous cries of the dying.

In those days, infection of the uterus and blood of mothers at childbirth was very common and often fatal. In many hospitals as many as one-half or even two-thirds of the mothers did not live to nurse their babies.

It must be remembered that this was before microbes were well known and before their part in disease was understood. Surgeons stood helpless before this mysterious and malignant foe, which so often made useless their best efforts. They were able to perform only a limited variety of operations with any hope of the patient's survival. Amputations were very common, especially after compound fractures, for it was felt that there was no other way to save the patient's life, though amputations themselves were frequently fatal.

Joseph Lister (1827–1912) and his antiseptic technique. In a previous chapter, it has been recited how the great English surgeon, Joseph Lister, pointed the way out. Lister was the first surgeon to disinfect his hands, instruments and dressings, and the first to demonstrate that infection of wounds may be prevented in this way.

The methods used to avoid infection at operation by Lister himself were very simple. He disinfected his hands, his instruments, ligatures, sponges, and dressings in carbolic acid solutions. At one time, in his earlier experiments, he was so impressed with the danger of infection from bacteria falling into the wound from the air that he employed a spray of carbolic acid about the wound during the operation. But later the spray was abandoned as unnecessary.

Not uncommonly, Lister performed operations in private homes. No special preparations were made beforehand, except the provision of trays and basins of carbolic acid solution, 1:20 and 1:40, which Lister often brought to the house himself. On his arrival, the instruments, which had been cleaned but not sterilized since the last operation, were placed in a tray of 1:20 carbolic acid, where they remained for some twenty or thirty minutes before the operation began. The sponges used to wipe out the wound during operation (marine sponges were used exclusively at that time) were soaked in the 1:40 lotion. When everything was conveniently arranged, the patient was brought in. While the anesthetic was being administered, the site of the operation, which had not been especially cleansed, was merely wiped with the 1:20 carbolic acid solution. Lister then "took off his coat, turned up his shirt sleeves, pinned an ordinary unsterilized huckaback towel over his waistcoat (for his own protection, not for that of the patient) and washed his hands in 1:20 lotion, or even what he called 'the strong lotion' (1:20 carbolic acid in 1:500 sublimate—bichloride of mercury—lotion), an ordeal that few of his adherents could endure. . . . He wore no mask or gloves." * Towels wrung out of carbolic acid solution were arranged about the field of operation. During the operation the hands of every one concerned were frequently dipped into the carbolic lotions and the sponges constantly conveyed small amounts of the disinfectant to the wound. Bleeding blood vessels were tied with catgut sterilized merely by soaking for a half hour in a 1:20 solution. The wound was dressed with a thick gauze pad, the part next the wound being saturated with carbolic acid.

Hospital operations were performed in the same way, under conditions of cleanliness no better than would be found in any decent home. Crude as this technique may seem to us, it must be remembered that it was a great advance over the habits of other surgeons of that day.

Surgical aseptic technique of today. What Lister accomplished with his crude method is now assured by a much more elaborate technique and a somewhat different plan. Now the use of chemical disinfectants is restricted as much as possible and infection of the wound is avoided by the use of instruments, dressings, etc., which have been sterilized by heat.

In a modern operation, the surgeon is assisted usually by several other persons, other doctors and nurses, each of whom have a definite task to perform during the course of the operation. The surgeon depends upon all of his assistants, but *especially upon the nurses*, for the perfection of the aseptic technique. They must be constantly alert to avoid contamination of instruments and supplies which have been sterilized. There must be undivided attention to the task, so that even the slightest mistake may be detected. The nurse should develop an acute "aseptic conscience" which will not permit her to overlook any break in technique, even though no one but herself is aware of it.

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* Sir Rickman Godlee, Lord Lister, Macmillan Co., 1917, p. 460.

Preparations of the surgeon and nurses. Before an operation, the surgeon and all his assistants "scrub up," that is, they *disinfect the hands and forearms* by a vigorous scrubbing followed by the use of a chemical disinfectant. Even though rubber gloves are to be worn, this must be carried out. This hand disinfection is not expected to make the skin sterile, but it is intended to make the hands as free of microbes as possible. The method is essentially the same as that used after contact with an isolated patient, only more prolonged and even more careful. The hands and forearms are scrubbed with a stiff sterile brush, using liquid soap and warm running water, for ten minutes. The nails are thoroughly cleaned with a sterile nail file. After the scrubbing, the hands and arms are rinsed in running water, then immersed in 70 per cent alcohol for three minutes. They are then dried upon a sterile towel. There is no short cut to this process of hand disinfection; the prolonged and thorough scrubbing is essential, and likewise the three minutes exposure to alcohol cannot be safely shortened.

A sterile *gown* is next put on with precautions not to touch the outside surface. Nurses put a *cap over the hair.* A sterile *gauze mask* is adjusted to cover the mouth and nostrils. The mask prevents droplets of bacteria-laden moisture, which might be expelled from the mouth or nose, from reaching the patient's wound.

The hands are dusted with sterile powder, then thrust into *sterilized rubber gloves.* Each pair of gloves is prepared previously by placing it in a folded towel or special muslin bag and sterilizing it in the autoclave.

Preparation of the patient for operation. The *field of operation,* that is, the part of the patient's skin at the place where the incision is to be made, is usually shaved the evening before the operation is scheduled. On the morning of the operation, this area is scrubbed with soap and water and rinsed with 70 per cent alcohol. Immediately before operation, the area is disinfected in one of several ways, depending upon the particular site and the preference of the surgeon. Two applications of tincture of iodine, followed by 70 per cent alcohol is a common method. Another agent frequently used is picric acid (5 per cent solution in

95 per cent alcohol). This is applied in two or more coats, immediately before operating.

Sterile towels are placed about this operating field, and sterile sheets are draped about the patient and the operating table, so that the actual area of skin in which the operative wound will be made is isolated as much as possible and protected from contamination.

Sterilization of instruments and other necessary materials. The *autoclave* is widely used for the sterilization of most instruments and supplies. Sometimes instruments are sterilized by *boiling in water for ten minutes.* A little bicarbonate of soda is usually added to the water to prevent rusting. The instruments must be *completely immersed* in the boiling water, otherwise they will not be sterilized. Knives, scissors, and other sharp instruments are usually boiled for a shorter period—about three to five minutes. Very sharp, delicate knives are not heated, but are sterilized by wrapping the blade in cotton soaked with 60 per cent alcohol, and then rinsed in sterile water just before use.

Hypodermic syringes are often sterilized by boiling for five minutes. The two parts of the syringe should be separated during the boiling and a little cotton should be placed in the pan to prevent breaking of the parts by bumping. Syringes may also be sterilized in the autoclave. The parts are first enclosed separately in gauze, then wrapped in paper or in a towel. In an emergency, hypodermic syringes may be sterilized by drawing acetone in and out several times. The barrel and plunger must then be warmed slightly over a flame (without touching any object) until the acetone is all evaporated and the glass is entirely dry.

Hypodermic needles demand great care. They should be washed and dried as soon as possible after use, so that they do not rust. They should be sharpened frequently. Like syringes, needles may be sterilized by boiling. Or dry, clean needles may be placed, point down, in a small test tube with a bit of cotton at the bottom. The tube must be plugged with a cotton stopper, then sterilized either in the autoclave or the dry heat oven.

Ligatures made of catgut or other material and used for tying off blood vessels, and sutures of silk thread or other substances used for sewing up the wound, are usually purchased already sterilized from the manufacturer. Sometimes they come in vials of alcohol in which they have been boiled. Sometimes the threads come wrapped in paraffined paper and have been sterilized in a dry heat oven. Some kinds of suture materials are boiled just before use.

Sponges, dressings, towels, and similar materials are all sterilized in the autoclave. The "sponges" now used are made of folds of absorbent gauze.

Dressing room technique. As much care is taken to avoid infection in the dressing room as in the operating room. The doctor and nurse doing the dressing usually wear sterilized rubber gloves. The soiled dressings are removed carefully and promptly sterilized. New sterile dressings, drains, etc., are handled with sterile forceps and protected from contamination with the most meticulous care.

REVIEW QUESTIONS—CHAPTER XX

1. Define *aseptic technique.* Explain why this technique is necessary in the care of the isolated patient.
2. What is meant by *isolation* of the patient? Must the patient necessarily be surrounded by a wall of some sort? Why not? Mention some diseases which require isolation.
3. Discuss the importance of cleanliness in the sickroom and tell how it may be secured.
4. Describe the proper dress and conduct of attendants in the sickroom of an isolated patient.
5. Why is it necessary to disinfect the hands after contact with the isolated patient? Describe a practical method of hand disinfection after such a contact.
6. What is meant by *concurrent disinfection?*
7. Discuss precautions necessary to protect food and drink from contamination.
8. Give a practical method, with all essential details, for the disinfection of (1) eating utensils, (2) patient's hands, (3) bath water, (4) bed clothing, bed linen, etc., (5) rubber articles, (6) enameled ware, (7) patient's toilet articles.
9. Why must clinical thermometers be disinfected? Explain the necessary steps for the sterilization of clinical thermometers in routine use.
10. Explain why it is necessary to carry out concurrent disinfection of the articles mentioned in Question 8. Explain the importance of disinfecting discharges from the body of the patient.
11. Give a practical method for the safe collection and disinfection of the following: (1) discharges from the nose, (2) sputum, (3) feces and urine, (4) pus.
12. What is meant by *terminal disinfection?* What is its importance as compared with concurrent disinfection? What procedures are neces-

sary to prepare a bed and room for use after occupancy by a case of communicable disease?

13. What is the importance of aseptic technique in modern surgery and obstetrics?
14. Describe conditions prior to the introduction of aseptic methods.
15. Review the work of Joseph Lister and its results.
16. Outline the antiseptic method used by Lister.
17. In general, how has Lister's technique been modified in present day practice? Discuss the importance of the part played by the nurse in modern aseptic surgery.
18. Describe a practical method for the disinfection of the hands of nurses and doctors in preparation for an operation. How are the surgeon and his assistants dressed? Why do they wear masks? Why rubber gloves? How are the gloves sterilized?
19. How is the patient's skin at the site of the operation disinfected? What measures are taken to isolate the field of operation from possible contamination?
20. Give practical methods, with all essential details, for the sterilization of (1) surgical instruments, (2) hypodermic syringes and needles, (3) sponges, dressings, and similar materials.

CHAPTER XXI

INFECTION THROUGH CONTAMINATED WATER, MILK, AND OTHER FOODS. INSECT-BORNE INFECTION

WATER-BORNE INFECTION

WATER IS found on the earth in the form of (1) *rain water*, (2) *ground water*, and (3) *surface water*.

Rain water contains very few bacteria, and if the cistern or other container in which it is caught is clean, it is quite safe to drink. A cistern should be constructed so that neither surface water nor ground water can get into it.

Ground water in the *deeper layers* of the earth contains no dangerous germs and very few bacteria of any kind, because the organisms have been filtered out as the water passed downward through the soil. When this deep ground water is brought to the surface by means of drilled artesian walls, an excellent supply of pure water is usually obtained. Natural springs which come from a considerable depth in the earth may also contain very pure water. Both wells and springs, however, must be protected from pollution at the surface of the ground.

The shallow dug well is dangerous. Unless it is properly constructed, it is liable to be polluted with waste matter from a near by privy or farmyard.

In order to be a safe source of drinking water, a shallow well must be located so that the land drains away from it, a watertight platform (for the pump) must be built over it to keep out surface drainage, and the upper portion of its walls must be made of concrete or other impervious material so that the water in the well comes *only from the deeper soil* (Fig. 54).

It should be remembered that shallow springs may be as dangerous as shallow wells, and for the same reasons.

Surface water, in the form of streams, ponds, or lakes, will naturally contain many harmless bacteria from the soil. In addition, a stream or lake in the neighborhood of a populous community is almost certain to be polluted with intestinal bacteria and contaminated with the germs of intestinal diseases. This results from the almost universal practice of dumping sewage into the nearest body of water. It is often necessary for a community to obtain its water supply from the same river or lake which receives the sewage of the city or neighboring cities. It is, therefore, only in rare instances that surface water can be used as a source of public water supply without employing some method of artificial purification.

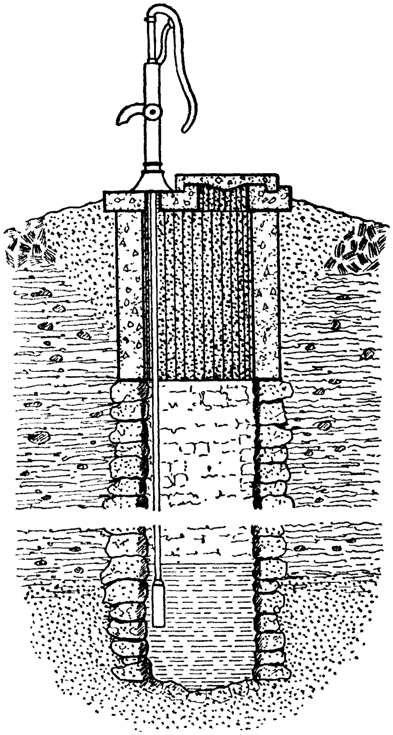

FIG. 54. A safe shallow well. The land drains away from the well. The base of the pump makes a water-tight connection with the concrete platform. The concrete construction of the upper part of the well, and the sand around it, protect against contamination from the surface of the ground and from seepage through the upper layers of the soil. The only water that can get into such a well comes from the deeper layers of water-bearing sand or gravel.

Diseases contracted from contaminated water. The kinds of illness which may be acquired directly from contaminated water are diseases of the alimentary tract, and these result from drinking of the water. The specific bacterial diseases which may be water-borne are *cholera*, *typhoid fever*, *paratyphoid fever*, and *dysentery*. *Amebic dysentery*, as well as bacillary dysentery may be carried by water. *Weils' disease* (infectious jaundice) may be contracted by swallowing water containing the causative spirochetes.

The life of disease germs in water is short, and they probably never multiply there. They are likely to remain alive longer in water containing a considerable amount of organic matter, and in water that is cool. Typhoid bacilli will live in polluted water for about one week. When epidemics are caused by contaminated water most of the cases appear within a few days of each other, indicating that all the patients were infected from the water at about the same time. And it is always found that the germs got into the water just a short time before the infection began.

Examples of water-borne infection.

The Broad Street well epidemic of cholera in London, 1854. The fact that disease may be acquired through drinking water was demonstrated long before much was known about germs. One of the classic examples of water-borne infection, and the first epidemic to be traced clearly to a polluted water supply, was the outbreak of cholera which occurred in St. James Parish in London in 1854. Although cholera was generally prevalent in London at the time, there were many more cases and more deaths in this particular parish than in neighboring areas. The outbreak was most intense between August 30 and September 9. During this period 530 individuals were attacked by cholera from which they later died. The investigations of John Snow and John York proved conclusively that the source of the epidemic was the polluted water of a certain well—the Broad Street well. Snow found that nearly all of the deaths occurred among persons who lived in the vicinity of this well and were accustomed to drinking its water. In the case of several persons who died of cholera in other parts of London, he was able to show that these persons had visited in the vicinity of Broad Street and had drunk the water of the Broad Street well. His careful inquiries revealed that workers in factories in the neighborhood who drank the water of this well suffered from typical cholera or from diarrhea, whereas those who drank water from other wells escaped the disease.

The probable manner in which the well was polluted was disclosed by York's investigations. He found that the drainage from a poorly constructed cesspool and privy of a house at No. 40 Broad Street was percolating into the well. Apparently the well-water had been polluted in this manner for some time, but it was only when the water was contaminated with a particular germ—the cholera spirillum—that cases of cholera appeared. Before the outbreak there had been a case of intestinal disease, undoubtedly an unrecognized case of cholera, at No. 40 Broad Street, and contamination of the well-water with the intestinal discharges from this patient in all probability accounts for the epidemic.

The cholera epidemic in Hamburg, 1892. This outbreak of cholera in Hamburg stands out in the history of sanitation as the most instructive example of the danger of polluted water and of the value of artificial purification of public water supplies. The epidemic was one of the most severe ever known. In the city of Hamburg, with a population of only 640,000 persons, there occurred during a period of two months about 17,000 cases of cholera, with 8,605 deaths. The cause of this outbreak was clearly traced to the contaminated public water supply. The citizens of Hamburg used the raw, unfiltered water from the Elbe River. This stream also received the sewage from the city, and therefore was always grossly polluted. At the time of the epidemic, investigators were able to demonstrate the presence of numerous cholera spirilla in the river water. These germs probably reached the water by way of the discharges of a group of immigrants from a cholera-infested country who were temporarily quartered on the river front.

While this very severe outbreak was going on in Hamburg, the neighboring city of Altona, directly adjacent to Hamburg on the Elbe River, escaped the disease, and this despite the fact that the water supply of Altona was also taken from the Elbe River at a point below Hamburg. But *the water for Altona was passed through a sand filter before it was distributed to the city.* The cholera germs and other bacteria were removed by the process of filtration, and thus the citizens of Altona were protected from infection. This dramatic demonstration of the effectiveness of water-filtration in preventing water-borne disease stimulated other communities to adopt methods of safeguarding their water supply, and led to the construction of water purification plants in many parts of the world.

Present danger of water-borne infection. Numerous examples might be given of outbreaks of typhoid fever and dysentery which have been traced to polluted water as definitely as the cholera epidemics just mentioned. Thirty years ago outbreaks of water-borne typhoid fever occurred frequently. In 1900, the death rate from typhoid fever in the United States was

35.9 per 100,000 population. At the present time (1935) the typhoid death rate is only 2.8 per 100,000 population for the country as a whole, and is still lower in some of the principal cities. This remarkable improvement has been brought about by the combined effect of several factors, such as better personal hygiene and purer milk, but it is due principally to the wider and wider adoption of water purification methods in municipalities, large and small, throughout the country. At the present time epidemics of water-borne disease are comparatively rare. They occur almost entirely in rural communities as a result of the use of contaminated well-water or spring-water.

Determination ot the quality of the water supply. In order to determine whether or not a particular body of water may be safely used as a source of drinking water, it is necessary to make a *sanitary survey of the watershed* to discover potential sources of pollution, and to conduct a *laboratory examination of the water.*

Laboratory tests upon the water include: (1) *a microscopical examination,* (2) *a physical and chemical examination,* and (3) *a bacteriological examination.*

It must be remembered that the appearance of the water to the naked eye does not give any reliable indication of its sanitary quality. A sparkling, clear water may nevertheless contain the germs of disease.

The *microscopic examination,* reveals the numbers and kinds of algae, protozoa, and other larger forms of microscopic life, and the nature of amorphous matter in the water. This examination often helps to explain objectionable odors and tastes which are due to the presence of large numbers of certain kinds of algae. Copper sulphate is added to the water in reservoirs to destroy these algae.

The *physical and chemical examination* includes the determination of such qualities as the color, turbidity, odor, temperature, and hardness, the content of free ammonia, nitrates, nitrites, and chlorine, and tests for poisonous minerals, such as lead. The presence of certain nitrogenous compounds in abundance suggests recent pollution of the water with sewage.

The *bacteriological examination* includes (1) *a quantitative analysis,* or *determination of the total count,* that is, *the number of bacteria per cubic centimeter* and (2) *a qualitative analysis,* or *tests for organisms of the colon group whose presence indicates intestinal pollution.*

The general method of counting bacteria has been outlined on page 201.

The qualitative test consists in the inoculation of *lactose broth fermentation* tubes with different amounts of the water sample. The appearance of gas after twenty-four to forty-eight hours incubation is evidence of the presence of some lactose fermenting organism, which may be presumed to be *Escherichia coli.* This presumption is confirmed by further tests, according to the standard procedures of water analysis established by the American Public Health Association and the American Water Works Association. *No attempt is made* in routine water analysis *to demonstrate typhoid bacilli* or other disease germs in the water. It is very hard to isolate them even when the water is known to be contaminated, and for practical purposes it is not necessary, since the presence of the more easily cultivated colon organisms is sufficient proof that the water has been polluted with intestinal wastes and is therefore unsafe to drink.

Qualities of a good water supply. A good water supply will be *sufficient in quantity* so that it may be freely used by all throughout the year. The water will be *free from all pollution with human or animal excreta,* and, therefore, *free from the germs of intestinal disease.* This necessitates the artificial purification of the water in most communities. Water which has been subjected to modern purification methods is almost entirely free of intestinal organisms, and indeed, contains very few bacteria of any kind.

In addition, good water will possess certain desirable physical and chemical properties which will tend to encourage its use for drinking and other purposes. It will be cold, clear, colorless, odorless, and have an agreeable taste. It will not be too hard, and it will be free of lead and other poisonous chemicals.

Artificial purification of public water supplies. Artificial purification may include the following processes: (1) *clarification and sedimentation,* (2) *filtration,* and (3) *chlorination.*

The water filters in use are of two kinds: (1) *the slow sand filters* and (2) *the rapid or mechanical sand filters.*

Slow sand filters are employed where the raw water is free from much turbidity, and preliminary clarification is not required. The filters consist of large, shallow, water-tight basins containing a bed of filtering material. This is made up of sand and gravel of different degrees of fineness, beginning with coarse gravel at the bottom and finishing at the top with fine sand (Fig. 55). The water is allowed to gravitate slowly downward through this bed of sand and the filtered, purified water is collected in drains beneath the filter.

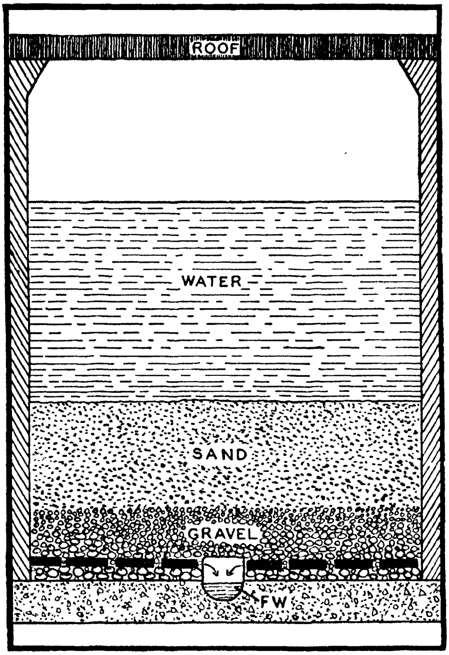

Fig. 55. A cross section of a slow sand filter. The roof is necessary in colder climates to prevent freezing of the water in winter. FW, filtered water conduit.

The process of filtration is not a simple straining. Its efficiency depends upon the formation of a gelatinous scum over the grains of fine sand in the upper layers of the filter. As the water slowly passes through, bacteria are caught in the scum as in a trap. Harmless saprophytic organisms become established in this sticky matter over the sand and effectively destroy parasitic bacteria that may be in the water. All pathogenic organisms and about 99 per cent of all bacteria are removed.

Rapid or mechanical sand filters are used where the water is originally muddy and colored, and where chemical coagulants are necessary. The filter bed is very much the same as in the slow sand filter. But *alum* is added to the water just before filtration, and the heavy, flocculent, jelly-like precipitate (aluminum hydroxide), which then forms, settles as a layer over the surface of the sand and serves in place of the naturally developed scum on the slow sand filter. The water is passed through the filter very rapidly and in this case the process is really a mechanical straining. These filters remove from 95 to 99 per cent of all bacteria.

Although both the slow and the mechanical sand filters will yield a safe water when properly operated, there is always a possibility of failure. It has become the practice, therefore, in most communities, to take the further precaution of adding a chemical disinfectant to the water after filtration and just before it is distributed to the mains. *Chlorine*, in liquid or gaseous form, is the agent used. The amounts employed vary from 0.3 to 1.0 part of chlorine per million parts of water.

Emergency methods of water purification. *Boiling.* The simplest and surest way to make water of doubtful purity safe to use is to boil it. The flat taste of boiled water may be removed by shaking it in a bottle or by pouring it from one vessel to another.

Chemical disinfection. The most widely used chemical agent for the disinfection of small amounts of water is *chlorinated lime*, or bleaching powder. This substance is suitable for emergency use by soldiers in the field, explorers, tourists, and campers. Rosenau gives the following directions: "Add one-half teaspoonful of chlorinated lime to one pint of water. Use one teaspoonful of this to 10 gallons, 36 drops to one gallon, or 9 drops to a quart. Let stand for at least 15 minutes."

MILK-BORNE INFECTION

It is hardly necessary to say that an abundant supply of pure, clean, cow's milk is essential in modern life. But milk is potentially our most dangerous food. It is difficult to get pure milk and to keep it pure. Infection through contaminated milk is common. Prevention of milk-borne disease is one of the most important, and one of the most difficult, problems of public health.

There are peculiar circumstances which make milk so liable to act as a source of disease. Milk is the only standard article of diet, obtained from animal sources, which is ordinarily consumed in the raw state (not cooked). Enormous quantities of milk and milk products—ice cream, butter, and cheese—are consumed every day. The larger part of this great quantity of milk is drunk by children, and they are more susceptible than adults to infection or poisoning through the digestive tract. Since milk is opaque, it may contain a great deal of dirt without giving any evidence of this pollution to the eye. Milk is itself a good culture medium for a great variety of micro-organisms, and pathogenic as well as saprophytic bacteria are able to *multiply* in it. The milk for large cities comes from hundreds of different dairies, passes through the hands of two or three different dealers, and is commonly transported for long distances, before it is delivered to the consumer. It is exposed to pollution from the moment it is drawn, and at any time during the long journey from the dairy to the table it may be contaminated with disease germs through the ignorance or carelessness of the numerous persons who are engaged in handling it. In view of all these circumstances, it is easy to see how milk may play so important a rôle in the spread of disease, and how serious is the problem of safeguarding the purity of milk supplies.

Sources of bacteria in milk. Milk as secreted in the mammary gland of the cow is sterile, but it is not possible to get this milk entirely free of bacteria even when the most elaborate precautions are taken to insure cleanliness in the milking. Some bacteria are always present in the milk ducts in the teats, and these are washed out when the milk is expressed. Milk obtained in the most cleanly manner possible will contain from a few hundred to a few thousand bacteria in every cubic centimeter.

When the milking is done without any effort at cleanliness, as is so often the case on the small dairy farm, the milk is certain to be contaminated with millions of micro-organisms. These organisms get into the milk from the dust and filth of the stable or barnyard, from the dirty flanks and udders of the cows, from the unwashed hands of the milkers, and from unsterilized milk pails. The only gesture of cleanliness ever made on many farms is to pass the milk through a strainer. This takes out the gross dirt—it is no novelty to find sizable masses of manure—but of course the bacteria are not removed.

Significance of the number of bacteria in milk. Freshly drawn milk, then, is never sterile, and unless great care is taken during the milking process to insure a scrupulous cleanliness, the milk is certain to be heavily inoculated with a great many micro-organisms from the very start. The number of bacteria in a sample of milk at any subsequent time will depend upon (1) the number of living organisms originally introduced during the milking, and (2) the temperature at which the milk has been kept. If the milk is cooled to about 50° F. (10° C.), or lower, immediately after it is drawn, and kept continuously cold, there will be only a gradual increase in the number of bacteria, because most of the organisms will multiply very slowly, if at all, at this low temperature. But if the milk is not cooled at once, or is allowed to become warm at any time, the bacteria in it will increase enormously within a very short time.

Counts of the bacteria in milk samples from dairies, collecting stations and pasteurizing plants, and in bottled milk as it is delivered, are of great value to public health officers, because these counts indicate the sanitary conditions which have prevailed during the production and handling of the milk. If the milk supplied by a certain dairyman contains a very large number of bacteria day after day, this is clear evidence of unsanitary methods. Fresh, clean milk, which has been kept cold, will have a low bacterial count; old and dirty milk will have a high count.

Danger in unsanitary methods of handling milk. Fortunately, the great majority of bacteria that get into milk are harmless saprophytes, and even very dirty milk, containing millions of these saprophytic organisms in every cubic centimeter, may be drunk by adults without appreciable harm. But such milk is, of course, very undesirable. It will sour much more quickly than clean milk. And *as food for infants dirty milk is dangerous*, even though it does not contain disease germs. It may be one of the contributing causes to the development of the severe diarrhea many infants suffer during the hot summer months.

The greatest danger in unsanitary methods of handling milk, however, lies in the opportunities such methods give for the contamination of the milk with disease germs. An ignorant or irresponsible dairyman, who does not keep his cattle free of disease, may sell milk from a sick cow, and this milk may contain germs which cause disease in human beings. Thus, some *animal diseases* may be transmitted to man through milk. More important is the possibility that the milk may become contaminated with the germs of *human diseases.* This may happen as the result of the use of unsterilized milking utensils, or the germs may be introduced into the milk by the hands of the milker or other persons engaged in its production or distribution.

Animal diseases transmitted to man through milk. *Tuberculosis.* This is the most important disease of cattle which may be transmitted to human beings. It is caused by the *bovine type* of *Mycobacterium tuberculosis.* Adult human beings are not susceptible to infection with this type of the tubercle bacillus, but young individuals (under 16 years of age, and particularly children under 5 years) may be infected with this organism by drinking raw milk from tuberculous cattle.

In the cow, the most common form of tuberculosis involves the lungs; but the sputum, instead of being expectorated, is swallowed, and the tubercle bacilli pass out of the body in the feces. Milk is contaminated with these organisms by fecal matter dropping into the milk pail from the dirty flanks or udder of the cow. Occasionally tubercle bacilli infect the mammary glands and so pass directly into the milk.

Undulant fever (*brucellosis*). Many cases of this disease have been traced to the ingestion of raw milk contaminated with one of the three varieties of the bacilli now classified in the genus *Brucella—Brucella melitensis*, *Brucella abortus*, or *Brucella suis.* These organisms are primarily pathogenic for goats, cows, and hogs, respectively, causing *contagious abortion* in these animals, but human beings also are readily infected through the skin or mucous membranes by contact with contaminated materials, or by drinking raw milk of goats or cows containing the germs. The disease in man contracted from contaminated goat's milk and prevalent for centuries on the island of Malta and in countries along the Mediterranean basin has long been known as Malta fever, but with the recognition of the widespread occurrence of a clinically similar disease elsewhere, the more general terms *undulant fever* or *brucellosis* (after Bruce who, in 1887, isolated the *melitensis* variety from Malta fever cases) are now commonly used. The disease in human beings is insidious in onset, and usually follows a protracted course, marked by successive waves of fever (undulating fever curve). (Chapter XXXVIII).

Human diseases spread through milk and milk products. The human diseases most frequently transmitted through contaminated milk are infections of the intestinal tract. The commonest is *typhoid fever*. *Diarrhea of infants* and *dysentery* may also be milk-borne. Less commonly, contaminated milk is responsible for infections of the upper respiratory tract with the germs of *septic sore throat, scarlet fever,* or *diphtheria.*

Examples of milk-borne disease. *Milk-borne typhoid fever.* The great majority of epidemics of typhoid fever occurring in cities in recent years have been caused by contaminated raw milk. When these epidemics are studied, circumstantial evidence usually points very clearly toward the milk supplied by a particular dairy as the source of the infection. Further investigation usually results in the discovery of a typhoid bacillus carrier, or an unrecognized case of typhoid fever among the persons employed at that dairy. Some epidemics have been traced to contamination of the milk by a person who was nursing a typhoid patient and at the same time acting as milker. Other outbreaks have been due to the use of water contaminated with typhoid bacilli for washing of milk utensils.

One of the most extensive of milk-borne typhoid epidemics occurred in 1927 in the city of Montreal, Canada.

During the period March 1 to June 28, 1927, 4,755 cases of typhoid fever were reported with 453 deaths. This is the most serious outbreak that has occurred in any large city for many years. Investigators found that neither the water supply nor sewage disposal systems were in any way responsible for the epidemic. But the evidence pointed clearly to a contaminated milk supply. It was found that a very large proportion of the cases occurred in persons who had drunk the milk distributed by a certain dairy company. Just exactly how or when the germs were introduced into this milk supply was not determined. It was found, however, that many of the 1,200 to 1,500 small farms which sent milk to this dealer were very unsanitary. Open privies were used on many farms, sewage-polluted water was used in the milk houses, and other unsanitary practices were common. Obviously, there were abundant opportunities for contamination of some of the milk by typhoid cases or carriers on the farms. The milk was supposed to be pasteurized, but there was inadequate supervision of pasteurizing plants. Investigators from the United States Public Health Service * concluded that the germs must have been introduced into the milk at some of the farms or collecting stations, and that these germs were given an opportunity to multiply during transportation, and that for some reason this contaminated milk was distributed to the city without being pasteurized.

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* "Report of the United States Public Health Service on the Montreal Typhoid Fever Situation." *Public Health Reports,* Vol. 42, 1927, p 1893.

A few typhoid fever epidemics have been caused by contaminated ice cream, butter and cheese.

Milk-borne septic sore throat. This is a severe type of sore throat caused by a variety of the pathogenic hemolytic streptococci called *Streptococcus epidemicus.* The disease may be spread by droplet infection or by other forms of personal contact, but it has frequently been carried by raw milk. Epidemics have usually been traced to contamination of the milk with streptococci from cases of sore throat among milkers. The usual course of events is about as follows. A milker infects the udder of the cow with streptococci from his own inflamed throat through his unwashed hands. The germs multiply readily in the warm milk left in the udder after milking, an abscess forms in the udder and for a number of days thereafter all the milk obtained from this animal is heavily contaminated and may cause a septic sore throat in persons who drink it in the raw state. Thus the cow serves as a kind of incubator for the streptococci which cause sore throat in human beings.

An extensive septic sore throat epidemic occurred in Massachusetts in 1928.*

In the town of "K," with a population of approximately 4000 persons, there occurred about 950 cases in the month of July, with 48 deaths. The milk supply of the town was not pasteurized. The largest number of cases were among persons who were supplied milk by Distributor A. This dealer secured his milk from several different dairies. In the milk from one of these dairies, Dairy D, streptococci were found, and at Dairy D one of the cows was found to have an acutely inflamed udder from which the *Streptococcus epidemicus* was isolated. Just prior to the outbreak of septic sore throat among the users of the milk, one of the milkers at Dairy D had suffered from a severe sore throat. Two of this man's children were also sick with sore throats. It is very probable that this man infected the udder of the cow and was primarily responsible for the epidemic.

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* LOMBARD, H. L. "Septic Sore Throat in 1928 in Massachusetts: Epidemiology." *Journal of Preventive Medicine,* Vol. 3, 1929, p. 81.

Methods for the production of safe milk. The first requisite for the production of good milk is *healthy milk cows.* It is especially important that tuberculosis in dairy cattle be recognized and that all tuberculous cows be eliminated from dairy herds. Much progress has been made in this direction in recent years, and the danger of milk-borne tuberculosis is now greatly reduced. Efforts should be made also to eradicate cows infected with *Brucella.*

Nothing contributes more directly to the production of good milk than the practice of a few elementary principles of *cleanliness* during the milking process. When the stables are clean, and the flanks, and udders of the cows are cleaned before milking, the chances for contamination of the milk with dirt and manure are greatly reduced. The simple procedure of washing the udders, then wiping them dry, just before milking, will in itself have a marked influence upon the number of bacteria that get into the milk. Contamination is still further reduced by the use of specially designed milk pails with narrow openings. The milking utensils should be clean, and preferably sterilized.

It is even more important that the milkers themselves should be healthy and trained to habits of cleanliness. Milking should be done with clean, dry hands. Men who are carriers of typhoid bacilli or other germs, or who are suffering from diarrhea, or sore throat, or other communicable disease, should not be permitted to handle milk.

A good dairy will have provision for *immediate cooling of the freshly drawn milk* to a temperature below 50° F. It is exceedingly important to keep milk continuously cold, so far as is possible, from the time it is drawn to the time it is consumed. Only bottled milk (in sterilized bottles) can be safe. It should be promptly delivered to the consumer in refrigerated vehicles.

Pasteurization and its value in preventing milk-borne disease. Experience has shown that although cleanliness in obtaining milk and care in handling it—using the methods just mentioned—help greatly toward the production of a safe milk supply, nevertheless *all raw milk is potentially dangerous.* It is not hard to see why this is so. The milk supply for most large communities is made up of a mixture of the milk from hundreds of small dairies and any one of a thousand persons might contaminate the supply somewhere in its progress from farm to consumer. The

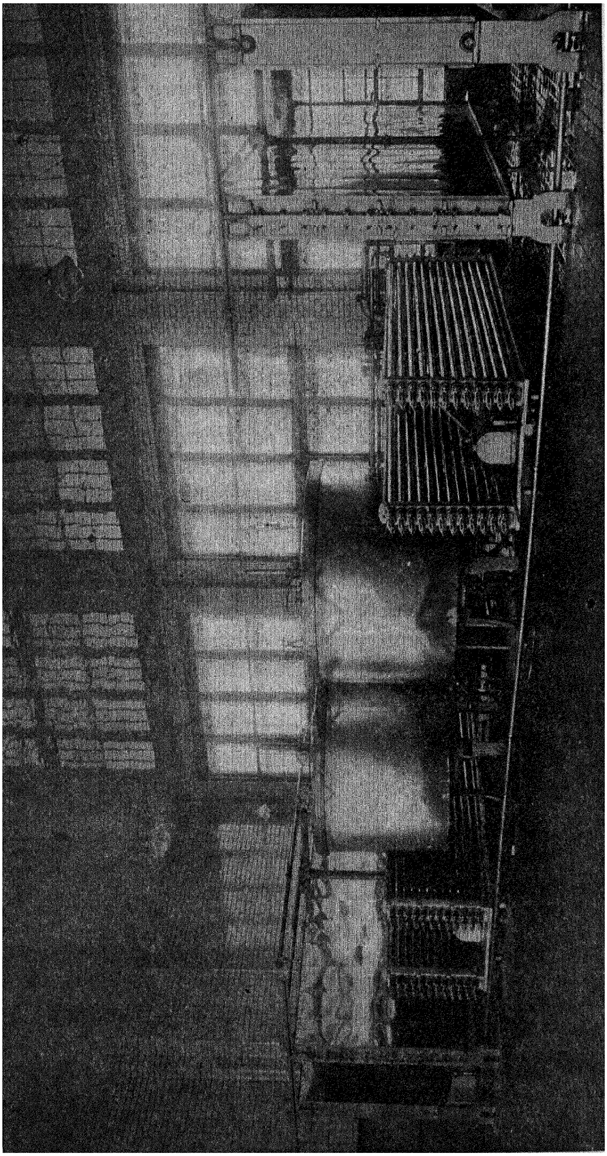

Fig. 56. A modern milk pasteurizing plant. The actual process of pasteurization goes on while the milk is held for a half hour period in the large tanks. The milk is heated to the pasteurizing temperature (142° F.) as it passes through the coil of pipes before it empties into the tanks. When the half hour pasteurization period is over, the milk travels through other pipes which cool it rapidly and the cold milk passes directly to the bottling machines in another room. The entire process is automatic and the milk is completely protected from contamination throughout. (Courtesy of the Pevely Dairy Co., St. Louis, Mo.)

existence everywhere of numerous healthy human carriers of disease germs make it almost inevitable that some lots of milk will be contaminated at times. Cows may also become diseased without its being known. Consequently a further safeguard is necessary. This is found in the process of heating the milk called *pasteurization.*

The milk is held in large vats, and continuously stirred, at a temperature of about 142° F. (61° C.) for a half hour, then from the pasteurizers it passes through cold pipes where it is *rapidly cooled.* It then goes directly to automatic bottling machines (Fig. 56).

Pasteurization of the entire milk supply of a community has been found to be a most valuable measure for the prevention of milk-borne disease. Pasteurization destroys all pathogenic bacteria and viruses which may be present in the raw milk. It does not *sterilize* the milk, but kills about 90 per cent of all the organisms present. It does not materially affect the quality of the milk as a food (except that it may reduce its vitamin content), and does not otherwise change its value.

Pasteurization will make even the dirtiest milk safer, but, of course, this is no justification for lax and unsanitary practices in the handling of milk at any stage of its production. Neither pasteurization alone, nor sanitary practices alone, can be depended upon to supply safe milk. Full protection from milk-borne disease can be secured only when all milk is obtained from healthy cows, in a cleanly manner, properly cared for before consumption, and pasteurized as a final safeguard.

Milk standards. Many communities in the United States have recognized, through local ordinances governing the sale of milk, two or more *grades* of milk, and have set up minimum standards which each grade must meet. The requirements are based upon conditions at the dairy farms and upon the result of chemical and bacteriological analyses of the milk. *Grade A pasteurized milk,* for example, is usually defined as milk from cows shown to be free of disease and produced and handled so that the total bacterial count at no time exceeds 200,000 per c.c. After pasteurization, and at time of delivery, this grade of milk should not contain more than 10,000 bacteria per c.c.

The use of the term *Certified* as applied to milk is limited to milk produced according to the strict requirements of the American Association of Medical Milk Commissions. It is intended to be the purest raw milk. Certified milk is not pasteurized, but is produced and handled with such great care that it can be delivered to the consumer with a bacterial count of less than 10,000 per c.c. Such milk is naturally more expensive than milk produced under ordinary conditions. So far as its health value is concerned, however, it has no advantage of any consequence over the best grade of pasteurized milk. It is doubtful whether the strict regulations which are supposed to govern its production are always adhered to in practice. At any rate, it is potentially a source of infection because it is not pasteurized.

INFECTION THROUGH FOODS OTHER THAN MILK

Food infection and food poisoning. Like milk, other foods may act as the vehicle of transfer for the germs of typhoid fever and other human infections, and they may occasionally transmit animal disease to man. Further, foods other than milk are frequently responsible for outbreaks of *food poisoning.* Aside from those foods, such as certain mushrooms, which are naturally toxic, *foods become poisonous for man as the result of contamination with certain micro-organisms.*

There are two important types of poisoning which may arise from contaminated food. These are: (1) the acute intestinal infection and intoxication popularly called "*ptomaine poisoning*" caused by the *Salmonella* group of bacilli or by certain strains of *Staphylococci*, and (2) the comparatively rare disease called *botulism*, caused by eating food contaminated with *Clostridium botulinum* and containing the powerful exotoxin produced there by this organism. These diseases are discussed in Chapter XXXV.

Danger from particular foods. The diseases mentioned above are not caused or spread by any particular kind of food. It is not the food itself, but the character of the germs that have gotten into it that determine the kind of disease it may bring about. Naturally the foods which are most liable to serve for the transfer of the germs of communicable diseases are those which are ordinarily consumed, like milk, in the raw state, without being heated or cooked in any way. The most important, besides milk, butter, cheese, and ice cream, are fresh vegetables and oysters and other shellfish.

Food poisoning is often caused by foods which have been *partially* cooked, *but have not been heated sufficiently* to destroy all the germs or poisons present. *Thorough cooking*, however, will make foods safe to eat. When we recall the innumerable opportunities for contamination of food by carriers of disease germs among cooks and other food handlers, it is clear that the custom of cooking most foodstuffs just before eating must prevent a great deal of illness.

Meat. Since meat is usually well cooked it is rarely responsible for disease. Raw or partially cooked meat, however, is dangerous. The general quality of market meat is protected in the United States by the Federal system of meat inspection at slaughter houses. Meat which has been passed by the Government inspectors is marked by a little purple stamp "U.S. INS'D & P'S'D." This stamp indicates that the meat is free from decomposed or diseased tissue. Only meat intended for interstate shipment is examined by Federal inspectors, however, and meat from animals locally slaughtered may not be safe.

One disease that has often been caused by home-killed meat is *trichinosis*. It is contracted by eating raw or insufficiently cooked *pork* infested with the *Trichinella spiralis*, a small roundworm. Hogs acquire this parasite from infested rats. The worms develop in the human intestines for about a week, then penetrate the intestinal walls, and migrate to the muscles in various parts of the body. There they form cysts, and the symptoms of the disease are due largely to these cysts in the muscles. Trichinosis in man is more common than is generally realized; it is thought to affect about 17 per cent of the general population. To prevent it all pork should be thoroughly cooked.

Raw or partially cooked pork and other meats and meat mixtures, such as sausage, have frequently been associated with outbreaks of bacterial food poisoning and botulism.

Fresh vegetables. It is easy to imagine how fresh vegetables might be contaminated with germs from the hands of the cook, and it is entirely possible that typhoid or dysentery might be caused by eating them. Rarely, cases of typhoid fever have been traced to the eating of raw celery, lettuce, and other fresh vegetables, obtained from gardens heavily manured with human excreta. Vegetables should be very thoroughly washed before they are put upon the table.

Shellfish. *Oyster-borne typhoid fever.* Among the shellfish used for food, oysters and clams are the most likely to carry infection because they are commonly eaten raw. Outbreaks of both cholera and typhoid fever have been caused by eating contaminated raw oysters. Before the danger was realized, and regulatory laws established, oyster beds were commonly situated in bays or inlets where the water was heavily polluted with sewage. It was also formerly the custom to fatten oysters by holding them in sewage-polluted, brackish water for a time just before they were put upon the market. Bacteria from the sewage, including typhoid bacilli, got into the bodies of the oysters, and these practices were responsible for several extensive epidemics of typhoid fever in the United States.

The danger of disease arising from oysters and other shellfish is slight, however, at the present time. There are now stringent Federal and state sanitary regulations which forbid the raising or fattening of oysters in polluted waters.

Canned foods. Canning involves a process of heating which is supposed to sterilize food, and, therefore, canned foods should be entirely safe. But occasionally the heating may not reach a sufficient temperature or may not be sufficiently prolonged to effect a complete sterilization, and after a time the food spoils. This is especially liable to happen to home-canned foods when the housewife does not fully understand the method of sterilization, or attempts to shorten the task by reducing the time of heating. The eating of spoiled canned food is probably often responsible for mild digestive disturbances.

Fortunately, none of the germs of specific human diseases, likely to be found in food, possess spores which might resist the heat of the canning process, with the single exception of the *Clostridium botulinum.* The spores of this germ are unusually resistant to heat, and most of the cases of botulism in this country have been caused by canned food. The majority of the outbreaks have been due to home-canned products. But botulism is very rare and the occasional occurrence of this disease should not destroy confidence in the safety of canned foods in general.

INSECT-BORNE INFECTION

The attention of the medical word was first attracted to insects as carriers of disease in 1893, when Smith and Kilbourne demonstrated that Texas fever, a malaria-like disease of cattle, is transmitted through the bite of ticks, these small insects acting as intermediate hosts for the germs causing the disease. Shortly after this came the observations of Ross and others which showed that malaria is carried by mosquitoes. These discoveries led to many investigations of the rôle of insects as carriers of germs, and it was soon apparent that they play a very important part in the spread of certain diseases.

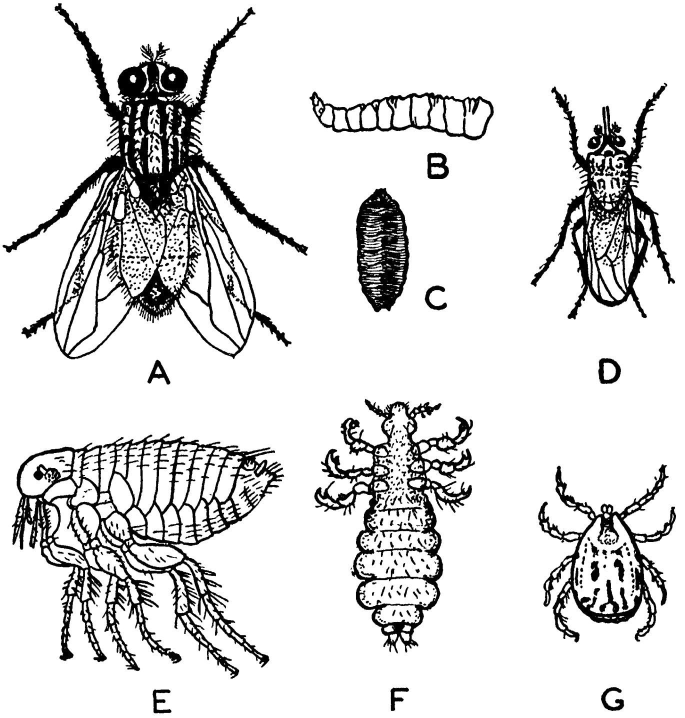

Fig. 57. Some insects that may carry germs. A, the house fly (dysentery and typhoid fever); B, the maggot, and C, the puparium of the house fly; D, the tsetse fly (African sleeping sickness); E, the Indian rat flea (plague); F, a louse (typhus); G, the wood tick (Rocky Mt. spotted fever).

Some general features of insect-borne infection. The kind of insects concerned in disease transmission are various: they in-

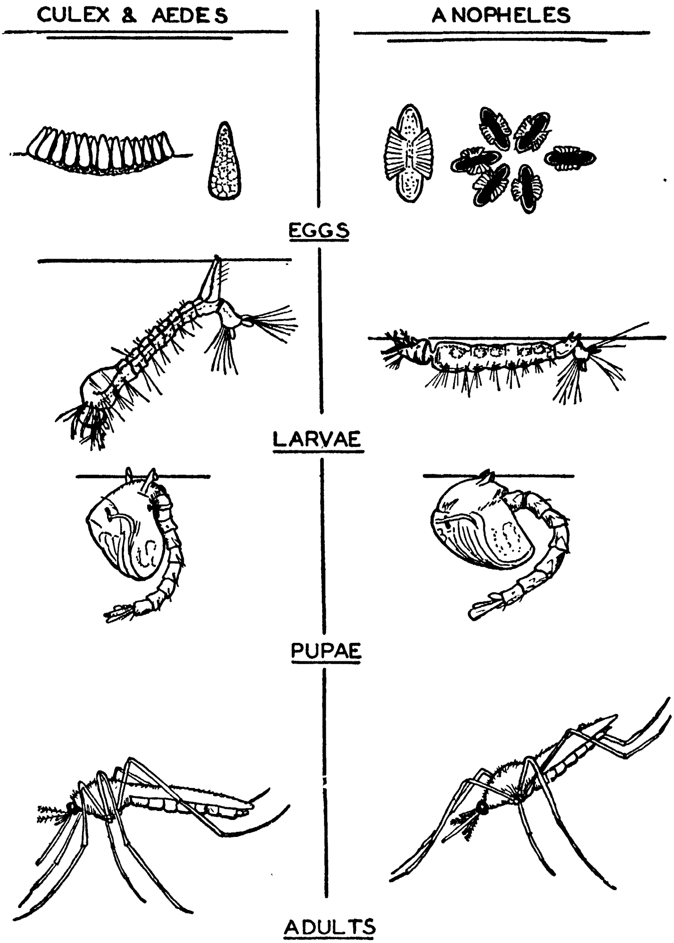

FIG. 58. Stages in the development of *Culex, Aedes*, and *Anopheles* mosquitoes.

clude flies, fleas, ticks, lice, and mosquitoes (Figs. 57, 58). All types of insects and arthropods which habitually suck the blood of human beings or animals are liable to act as *vectors* (carriers) of disease germs. In most cases, however, a particular disease is spread only by a particular kind of insect. Thus, malaria is carried only by mosquitoes of the genus *Anopheles,* while yellow fever is transmitted by *Aedes* mosquitoes, and sleeping sickness spreads only by the bites of tsetse flies, and so on. It is remarkable that the insects are not usually harmed at all by the germs they carry, despite the fact that in many instances these organisms multiply and undergo complicated changes as part of their life-cycle of development within the bodies of their insect hosts.

Insects act in some cases merely as (1) *agents for the mechanical transfer of germs,* but in most cases they serve as (2) *intermediate hosts for the germs,* and the sole or the chief means by which the infection is spread. In the latter instance, the germs undergo a series of changes within the body of the insect, and a definite interval of time must elapse after the organisms have been taken in before these changes are complete, and the insect becomes capable of transmitting the infection. This is spoken of as *biological transmission* of disease by insects.

PRINCIPAL INSECT-BORNE DISEASES: MECHANICAL TRANSFER

DISEASE	CAUSATIVE ORGANISM	INSECT CARRIER	DETAILS OF TRANSFER
Typhoid fever Cholera Dysentery, etc.	Eberthella typhi Vibrio cholerae Shigella dysenteriae etc.	House flies Cockroaches	Mechanical transfer of germs from excreta to food
Plague	Pasteurella pestis	Fleas of rats and other rodents	Germs in flea feces transferred through bites from infected rat or other rodent to man
Tularemia	Pasteurella tularensis (Bacterium tularense)	Horse flies, ticks, deer flies and other blood sucking insects	Deer flies may carry germs from rabbits dead of tularemia to man. Rabbit louse or wood tick may spread infection from rabbit to rabbit. Bedbugs may carry the germs

Since most of the insect-borne diseases are spread only by certain insects, we naturally find these infections confined to areas in which the insect hosts abound and human or animal carriers of the germs are numerous. Thus, malaria is prevalent only in places where there are many persons carrying the parasites (who, therefore, act as a reservoir of the infection) and where *Anopheles* mosquitoes are always present.

Principal insect-borne diseases. The table on page 288 gives the principal human diseases which may be spread as a result of *mechanical transfer* of the germs by insects. It will be noted that the germs carried in this way are *bacteria.*

The table on this page shows the principal diseases in which insects act as intermediate hosts for the causative organisms.

PRINCIPAL INSECT-BORNE DISEASES: BIOLOGICAL TRANSMISSION

DISEASE	CAUSATIVE ORGANISM	INSECT CARRIER
Malaria	Protozoa of the genus *Plasmodium*	Mosquitoes of the genus *Anopheles* (*Anopheles quadrimaculatus*, etc.)
Yellow fever	Filtrable virus	Mosquitoes of the genus *Aedes* (*Aedes aegypti*, etc.)
Dengue	Filtrable virus	Mosquitoes of the genus *Aedes* (*Aedes aegypti*, etc.)
Filariasis	Filaria (roundworms)	Mosquitoes of the genus *Culex* (*Culex quinquefasciatus*)
European Typhus fever	Rickettsia	Lice (*Pediculus humanus*, variety *corporis*, and *capitis*). (Human body and head lice)
Trench fever	Rickettsia	Lice (*Pediculus humanus*)
European relapsing fever	Spirochetes of the genus *Borrelia*	Lice (*Pediculus humanus*)
Asiatic relapsing fever		
Relapsing fever (African tick fever)	Spirochetes of the genus *Borrelia*	Ticks (*Ornithodoros moubata*)
Rocky Mountain spotted fever	Rickettsia	Ticks (*Dermacentor andersoni*)
Endemic Typhus fever	Rickettsia	Rat Fleas (*Xenopsylla cheopis*, etc.)
African sleeping sickness	Protozoa of the genus *Trypanosoma*	Tsetse fly (*Glossina palpalis; Glossina morsitans*)
South American Trypanosomiasis (Chagas' disease)	Protozoa of the genus *Trypanosoma*	Assasin bugs (*Triatoma megista*)

Most of the germs here concerned are protozoa, spirochetes, or filterable viruses, rather than bacteria. These organisms and viruses undergo complex changes within the bodies of their insect hosts, and it is only after this development is complete that the disease may be transmitted to new victims by the bite of the insect.

Prevention of insect-borne infection. Successful prevention of an insect-borne disease requires an intimate knowledge of the life history and habits of the insect involved, as well as a thorough understanding of the causative organism and the nature of the disease. The most effective method of controlling this kind of disease is usually to *prevent the breeding of the insect carrier* Thus, malaria and yellow fever may be combatted most successfully by destroying the eggs and young forms of mosquitoes, and by eliminating as far as possible the breeding places of these insects.

REVIEW QUESTIONS—CHAPTER XXI

1. How many, and what kinds of bacteria are likely to be present in (1) rain water, (2) ground water, and (3) surface water?
2. Why are shallow wells and springs dangerous as sources of water supply? What are the essential features of a properly constructed, sanitary shallow well?
3. Why is artificial purification usually necessary before surface water can be safely used as a public water supply?
4. Name diseases which may be contracted directly from contaminated water. Discuss the life of disease germs in water and characteristic features of water-borne epidemics.
5. Describe two well known epidemics of water-borne cholera. What other disease was frequently water-borne 20 or 30 years ago? What is the present danger of water-borne infection?
6. What must be done to determine the sanitary quality of a particular water supply? What is the purpose and value of the microscopic and the physical and chemical examination of water?
7. What constitutes the bacteriological analysis of water? Explain briefly the method and the purpose of: (*a*) the quantitative analysis and (*b*) the qualitative analysis.
8. Describe the qualities of a good water supply.
9. What three processes may be included in artificial water purification methods?
10. Describe briefly the construction and operation of (*a*) slow sand filter, (*b*) rapid or mechanical sand filters.
11. How and why is chlorination of water carried out?

12. Describe practical methods for purification of small amounts of water.
13. Discuss the importance of a pure milk supply and give reasons why milk is so liable to be a source of infection.
14. What are the principal sources of bacteria in milk?
15. What factors determine the number of bacteria in a sample of milk at any time after milking? Explain why bacterial counts reveal the condition under which milk has been produced and handled.
16. What dangers are associated with the use of dirty milk containing many saprophytic organisms?
17. Name and discuss briefly two animal diseases which may be transmitted to man through milk.
18. Name six human diseases spread through milk and milk products.
19. Describe how typhoid fever may be transmitted by milk.
20. What is the cause of septic sore throat? Describe how the disease may be spread by milk.
21. What measures are essential for the production of safe milk?
22. Mention some of the means by which contamination of milk at the time of milking may be reduced, and describe how the milk may be kept in good condition until it is consumed.
23. What is the process called pasteurization? Explain its value in preventing milk-borne disease. Should pasteurization alone be depended upon to make milk safe?
24. What are the usual standards recommended for Grade A pasteurized milk? What is certified milk?
25. What are the two important types of food poisoning and what kinds of bacteria are responsible?
26. What kinds of foods are most likely to transmit infection and why? What is the effect of thorough cooking?
27. Discuss infection from meat. Explain how trichinosis is contracted. How is it prevented?
28. How may fresh vegetables carry infection?
29. Describe how typhoid fever may be transmitted by raw oysters.
30. In general, what is the probability of infection from canned foods? What rare disease may be caused by improperly canned food?
31. What discovery first called attention to insects as carriers of disease?
32. In what two ways may insects act as carriers of germs? What is meant by biological transmission of disease by insects?
33. Discuss some general features of insect-borne infection.
34. Name three important diseases, the germs of which may be carried mechanically by insects. Give the insect concerned, the cause of the disease, and explain briefly how the germs are transferred in each case.
35. What is meant by intermediate hosts? Explain why there is a definite interval of time after the germs have been taken into the body of the insect before the insect can spread the disease.
36. Name thirteen diseases which are carried by insects acting as intermediate hosts and give the causative organism and the insect concerned in each case.
37. What is the most effective way of controlling insect-borne infections?

PART FOUR

INFECTION AND RESISTANCE

CHAPTER XXII

MICROBES AND DISEASE

DEFINITIONS

WHEN living bacteria, or other microscopic organisms or viruses, *enter the tissues, multiply there, and cause injury to the body cells,* we say an **infection** has occurred.

Infection should be distinguished from *contamination.* A contaminated object is one which carries or contains bacteria, especially disease germs. The dishes used by a patient with typhoid fever, for example, may be *contaminated* with the germs of that disease, but could not be said to be infected. Often the hands are contaminated without being infected.

Infectious disease. *Any disease resulting directly from an infection with a microscopic organism* is called an *infectious disease.* Thus tuberculosis, measles, typhoid fever, pneumonia, and tetanus are all infectious diseases, though each is a distinctly different kind of illness, because the existing cause of the disease in each case is an infection with a living germ or virus.

Sometimes the word *infectious* is used in a more special sense to mean "liable to be communicated," thus having the same significance as *communicable.* For the sake of clarity of thought, however, it is better to use *infectious* only in the general sense first given above.

A distinction should be made between the *infection itself*, that is, the entrance of the germ and the injury to the body tissues, and the *clinical disease* it may bring about. The infection always occurs some hours or days, and often weeks and even months, before symptoms of the disease appear. It is usually about ten days, for example, after typhoid germs have entered the intestine before the disease sets in. There are also some cases in which an infection causes so little damage that no symptoms are produced. For example, tubercle bacilli infect a small area of the lungs in many individuals who never become actually ill with tuberculosis.

It is necessary, therefore, to distinguish between tuberculous infection and tuberculous disease.

Communicable disease. A communicable disease is *one which may be transmitted naturally from one individual to another.* All communicable diseases are infectious, that is, they are all caused by microscopic organisms or viruses, and the germs concerned in each case have the capacity to pass in some way from one person to another during the everyday comings and goings of men.

A few of the infectious diseases are not naturally communicable. Tetanus (lockjaw), for example, which is caused by infection of a wound by *Clostridium tetani,* is not communicable, because this organism has no natural means of getting from the infected wound to a similar wound in another person. Therefore, cases of tetanus do not arise by contact with other cases.

The great majority of infectious diseases are communicable, however, though by no means all in the same degree. Mumps, measles, smallpox, and influenza, for example, are highly communicable; that is to say, the germs of these diseases pass readily from one person to another. Many other diseases do not spread so easily. In general, the diseases most readily communicated are those in which the microbes attack the upper respiratory tract, and are transmitted to other persons through the discharges from the nose, mouth, and throat.

Since even the diseases which are not naturally communicable can be transmitted by artificial transfer of the germs, the expression *communicable diseases* is generally used to include all the infectious diseases.

Contagious disease. The word *contagious* is sometimes used to mean the same as *communicable.* But *contagious* refers to *direct contact,* and, in a strict sense, a contagious disease is *one which is acquired only through personal contact.* Such diseases as measles, mumps, chicken-pox, and influenza are properly called contagious, because they are always communicated by personal contact with the patient. Typhoid fever, on the other hand, should not be called contagious because there is little danger of contracting the disease through a single contact with a patient, whereas the germs are often transmitted through contaminated water or milk or by other remote means. This distinction in the use of the term contagious cannot be carried out consistently.

however, and it is best to avoid the word entirely. *Communicable* is much to be preferred, for its meaning is clear.

Endemic, epidemic, pandemic disease. A disease is said to be *endemic* when a small number of cases occur constantly among the population of a community. Thus, measles is endemic everywhere in the United States. Cholera is endemic in India, but not in this country.

When an endemic disease flares up, as it were, and an unusually large number of cases develop within a certain community within a short time, we say the disease has become *epidemic*. When an epidemic becomes very widespread it is spoken of as a *pandemic*. During 1917–1919 influenza was at first epidemic in certain places, then became pandemic, spreading over virtually the entire world.

KINDS OF PATHOGENIC MICROBES

The **pathogenic** (disease-producing) **microbes** which we encounter in connection with illness in human beings and animals have one outstanding characteristic—they are capable of developing (though in some cases to a limited degree only) *within the tissues* of the living body. These interior parts, beneath the surface of the skin and mucous membranes, are free of microorganisms in health, and among the innumerable kinds of bacteria, fungi, and protozoa, it is only the relatively few species we call pathogenic that are able to gain a foothold there.

These pathogenic organisms are not all capable of infecting the body with the same ease or in the same circumstances. For practical purposes, it is useful to distinguish between two classes of disease-producing microbes: (1) the "opportunists," and (2) the "true pathogens."

Infection by "opportunists." *The "opportunists"* are those organisms capable of producing disease only when given a special opportunity to enter the body tissues through an accidental injury to the skin or mucous membranes, or when the natural resistance to infection is abnormally low. The germs of this kind do not invade the tissues of healthy individuals, but if they are introduced mechanically, through a wound, they may produce a severe or even fatal infection.

Tetanus is an example of an "opportunist" infection. The spores of the tetanus germ exist in the soil and become very widely distributed over all sorts of dirty objects. The bacillus is a saprophyte. It has no power to invade the body by itself. But it may be carried mechanically, with dirt, into an accidental wound. If conditions in the wound are favorable, it may multiply there to a limited extent. In so doing it secretes a powerful exotoxin which causes the fatal disease we call tetanus.

Among the bacteria inhabiting the healthy skin and mucous membranes there are many species which must be classed as "opportunists." The outstanding example is the *Staphylococcus aureus.* This organism exists normally on the intact skin and there it does no harm. But if it is given an opportunity to enter the deeper tissues through a cut or other break in the skin, an infection will result.

There are many examples of "opportunist" infections in persons whose natural resistance has been greatly reduced by preexisting disease or other abnormal condition. Infections of more or less severe character in the mouth, throat, lungs, appendix, urino-genital organs, and elsewhere, are often caused by organisms which are ordinarily harmless, but which have become pathogenic when a local injury, or a very low vitality of the individual, permits them to pass into susceptible tissues without resistance.

Infection by "true pathogens." In contrast to the "opportunists," the "true pathogens" are able to invade the tissues of healthy individuals through some inherent power of their own. There are only a few species of "true pathogens." Each of them brings about a characteristic illness in one victim after another.

The most common and familiar *communicable diseases,* such as diphtheria, tuberculosis, typhoid fever, syphilis, and meningitis, are each caused by a particular kind of "true pathogen" which will always produce the same kind of disease in susceptible persons.

Differences. No hard and fast line can be drawn between an "opportunist" on the one hand and a "true pathogen" on the other. But it is often very helpful to make this distinction. "Opportunist" infections differ in a number of ways from the characteristic diseases caused by the "true pathogens." One difference is that "opportunist" infections are not ordinarily communicable. From the point of view of the individual, "opportunist" infections are very important, and must be guarded against by careful disinfection of wounds, and by efforts to avoid unhealthful habits of living and weakening diseases which may lower resistance to infection. But from the point of view of public health, they are much less important since they cause diseases which do not ordinarily spread to others. Another important difference, which holds in most cases, is that recovery from "opportunist" infections, such as an appendicitis or secondary bronchopneumonia, does not usually make an individual more resistant to a similar infection, whereas a person who has survived an attack of most of the common specific diseases, such as typhoid fever, is rendered more or less permanently immune to that disease.

FACTORS GOVERNING THE START AND COURSE OF AN INFECTION

There is no microbe that will always produce disease in all kinds of living things. Most germs are pathogenic for human beings only, or for certain species of animals or plants only. The disease-producing power of a micro-organism must always be thought of in relation to some particular *host* * (man, animal or plant).

Furthermore, an organism which is pathogenic for some human beings or some individual animals will not necessarily produce disease in other persons or in other animals of the same species. Many factors determine whether or not a pathogenic organism can bring about an infection in any particular case. *The mere presence of the germ on the body surfaces is not necessarily followed by the development of the disease.*

There are four principal factors which determine the start and the course of an infection. These are: (1) *the place the organisms enter the tissues*, (2) *the number, or dose, of organisms entering*, (3) *the virulence of the organisms*, and (4) *the resistance of the individual host.*

Place the organisms enter the tissues. The particular tissues with which a germ first comes into contact, that is, its *path of entrance* into the body, often determines whether or not an infection will take place. The germs of typhoid fever and other intestinal infections can produce the typical disease only when they reach the intestines through the mouth. On the other hand, the germs of diphtheria, meningitis, gonorrhea, and many other diseases may be swallowed without harm. If typhoid bacilli were rubbed into the injured skin, they would cause only a slight inflammation and typical typhoid fever would not be brought about, but if anthrax bacilli were rubbed in they would produce a characteristic local lesion. Each of the "true pathogens" invades the body by a particular route, and unless it reaches the accustomed path of entrance, infection may not occur.

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* The *host* is the person or the particular animal or plant which supports a parasite. The host "entertains" bacteria or other parasitic organisms, as it were, at its own expense.

Number, or dose, of organisms entering. Obviously, the greater number of germs entering, other factors being equal, the greater likelihood that infection will occur, and the more severe the resulting disease is likely to be. The normal, healthy body possesses some natural defences against invasion by germs, and the dose of microbes entering must be sufficient to overcome this resistance. The more powerful the germs and the more susceptible the individual, the smaller the dose required. It has been claimed that a single anthrax bacillus can cause anthrax in a very susceptible white mouse. The matter of dose probably has an important influence in many cases of human infection. Tuberculosis, for example, is not usually acquired by adults solely from occasional contacts with a tubercular patient, but sometimes it is contracted by persons who live intimately for some time with an individual having the active disease, probably because in these circumstances they cannot avoid exposure to a heavy dose of the germs.

Virulence of the organisms. Virulence is a general term meaning *the disease-producing power of an organism.* Virulence must be thought of as a property of a pathogenic organism itself, as something inherent in the nature of the microbe. A virulent organism is one that can (1) multiply within the tissues of the body, or (2) poison the body. Most pathogenic organisms can grow readily within certain regions of the body, at least, and can poison the tissues as well.

Degrees of virulence and variation in virulence. Some species of pathogenic bacteria are naturally more virulent than others. Streptococci, for example, always tend to produce a more widespread and dangerous infection than staphylococci, that is to say, the streptococci are usually more virulent than the staphylococci. It is a very serious matter when the eyes are infected with the gonococcus, because this is a very virulent germ, whereas an inflammation caused by a less powerful organism is not greatly to be feared.

Virulence differs in degree not merely with different species of disease germs, but it differs in different cultures or strains of the same kind of germ. In other words, virulence is not a constant property, but varies in degree according to the condition of the particular strain of organisms concerned.

Decrease of virulence. A pathogenic organism tends to *decrease* in virulence when it is removed from its natural environment in the bodies of man or animals and forced to live under artificial conditions in laboratory culture media, and particularly if it is exposed to abnormal temperatures, to drying, or to other unfavorable conditions. A strain that has been weakened in virulence is said to be *attenuated.*

Many pathogenic bacteria, for example the pneumococci, decrease greatly in virulence and may become entirely nonvirulent when cultivated in the laboratory for some months. Other germs do not lose their disease-producing powers merely as a result of artificial cultivation, but, on the contrary, may retain their original virulence even after years in the laboratory. They may be attenuated, however, by a number of special methods. They may be grown at a temperature several degrees above the optimum for the species, or cultivated in the presence of weak antiseptics, or they may be subjected to partial drying. Such artificial methods of reducing virulence are of great importance in connection with the preparation of vaccines for the prevention of anthrax, rabies, and other diseases.

Sometimes the continual passage of a germ or virus through one species of animal will reduce its virulence for other species. Cowpox virus (used for vaccination against smallpox) is smallpox virus which has been reduced in virulence for human beings by cultivation in the tissues of the cow.

Increase of virulence. The virulence of any strain of pathogenic bacteria is *increased* when the organisms are passed frequently from one susceptible individual to another. For example, the virulence of pneumococci for white mice may be increased many times over by passing the organism directly from one mouse to another through a series of five or six animals. In this way a strain of these organisms which has originally almost no effect upon mice, even in large doses, may often be made so virulent that the inoculation of an amount equivalent to no more than one-millionth of a cubic centimeter of a broth culture will kill the animal in forty-eight hours.

Important facts about virulence. There are two facts concerning virulence which have an important practical bearing on methods of preventing communicable diseases.

(1) *An organism is, as a rule, most virulent when freshly discharged from the body.* The closer the personal contact with a patient or carrier, the greater the danger of infection, not merely because the dose of germs received may be greater, but because the bacteria in the pus, sputum, or other material freshly discharged from the patient's body are likely to be in their most highly virulent state. The great importance of disinfection of these discharges is obvious.

(2) *Virulence is increased when germs are rapidly passed from one person to another.* Communicable diseases are much more likely to occur among groups of persons who are crowded together in schools, institutions, or army camps, than among the general population. One reason for this is that the close personal contact permits the germs to be passed about readily and they reach one susceptible individual after another in rapid succession. This rapid transfer may increase the virulence of the organisms to such a degree that a mild endemic disease becomes converted into a serious epidemic one.

Resistance of the individual host. The final factor which determines whether or not an infection will take place, and the severity of the disease if it does occur, is the degree and character of the resistance offered to the invading organisms by the individual host. Once an infection has started, the body tissues do not submit to injury without protest. On the contrary, after a variable time, there begins a more or less profound reaction. This reaction is a sign that the body is defending itself against the harmful effects of the germs, and it is the attempt to get rid of the offending microbes, to repair the damage they have caused, and to neutralize or destroy their poisons, that brings about the signs and symptoms of the clinical disease.

There can be no infection, of course, if the individual is entirely immune, *i.e.* not susceptible to the particular germ.

Summary. The relationship between the factors of infection hich have just been outlined may be expressed in the formula uggested by Dr. Theobald Smith:

$$D = \frac{NV}{R}$$

) means *disease*, N means *number* (of the germs), V means *irulence* (of the germs), and R means *resistance* (of the host). he formula signifies that, in any case of infection, it is the total isease-producing power of the organisms (which depends upon heir number and virulence), balanced against the resistance of he infected individual, that determines the nature and severity of he disease.

GENERAL CHARACTERISTICS OF INFECTIOUS DISEASES

Varieties of infection. *Acute or chronic infection.* An *acute* nfection is one which runs a rapid and severe course, then termiates rather abruptly. Lobar pneumonia is a typical acute disase. It begins suddenly, the patient is very ill for a few days, hen, if death does not occur, recovery is usually rapid. The ajority of the common infectious diseases are typically acute.

Chronic diseases are those which run a slow course, usually ith comparatively mild symptoms, over a period of weeks, onths, or years. Tuberculosis in adults is in most cases a typical hronic infection. So, also, is leprosy and syphilis. *Very often n infection at first acute, later become chronic.* Gonorrhea, for xample, begins as an acute inflammation of the urethra, but later ecomes a chronic infection involving the deeper parts.

Primary or secondary infection. By the *primary* infection is eant the first or original infection which makes a person ill. A *econdary* infection is one which follows as a complication of the riginal disease. Secondary infections are commonly due to oranisms of the "opportunist" type. These infections are often ery serious, for the patient is in a weakened condition. One f the commonest causes of death is an infection of the lungs bronchopneumonia) which follows diseases due primarily to ther germs, as in influenza, measles, and whooping cough. Secndary infections of the middle ear, mastoid, meninges, lungs, or ther parts, are often more to be feared in cases of measles, scarlet ever, and many other diseases, than the primary infection itself.

Local or generalized infection. A *local* infection is one confined to one spot or area in the body. A boil is a local infection. Infections are said to be *generalized* when they involve the whole body. A generalized infection, such as is sometimes caused by the staphylococci, in which there are many separate abscesses located in various parts of the body, is called *pyemia.* When bacteria are present in the circulating blood, as for example, in the early stages of typhoid fever, the condition is called *bacteremia. Septicemia* is a term used by physicians to designate an infection which involves the blood stream. In many septicemias bacteria are actually multiplying in the circulating blood; this is the condition called "blood poisoning" in popular language. There is no sharp distinction between the terms *septicemia* and *bacteremia.* When the body is poisoned by a toxin carried about by the blood, as in diphtheria, the condition is called *toxemia.*

Focal infection. This term is applied to a local infection which acts as a *focus,* or center, from which germs spread to set up infection in other parts of the body. Abscesses about the teeth and chronic inflammation of the tonsils, and similar conditions, are common focal infections. From these centers of infection germs may enter the lymph or blood stream and be carried about. This is one of the ways in which microbes may reach the joints, and the heart valves, and set up arthritis and heart disease.

Latent infection. An infection is said to be *latent* when it is not progressive, but is quiescent, dormant, hidden. There may be no symptoms whatever, though the germs are still present in the body. In the course of syphilis and tuberculosis, there are often long periods during which the infection remains latent and the patient is free of symptoms, yet the presence of living germs in the body is manifested some time later when signs and symptoms of the disease again appear. There are similar latent periods in brucellosis and in tularemia.

Specific and nonspecific infection. A *specific* infection is one caused in all cases by a particular bacterium, such as syphilis, tuberculosis, and other infections with "true pathogens." Many infections, as, for example, inflammations of wounds, of the lungs and middle ear, are *nonspecific* in character, the germs present depending upon just what "opportunists" happen to get in.

Mixed infections. An infection is said to be *mixed* when microbes of two or more kinds are responsible. Most "opportunist" infections are mixed, two or three different species of organisms being present in the infected tissues.

An outstanding example of a disease caused by two different organisms apparently operating in symbiosis is Vincent's angina (Fig. 68). Such mixed infections are probably more common than is generally realized. In many diseases for which the primary cause is a filtrable *virus*, such as influenza and common colds, certain bacteria are likewise present, and the activities of these accompanying bacteria account in large part for the usual course of the illness.

Typical course of infectious diseases. During the course of any infectious disease, there are three more or less distinct phases. Before the disease actually begins there is: (1) the *period of incubation;* this is followed by (2) the *period of illness,* which merges into (3) the *period of convalescence,* during which the patient recovers his normal health.

Incubation period. This is a very important feature of all germ diseases. The incubation period is defined as *the interval of time between the entrance of the germs into the body and the appearance of symptoms of the disease.* Even though the organisms are very virulent, and the dose very large, there is always a lapse of some hours, days, or weeks, after infection has occurred, before the illness begins. Just what happens during the incubation period is not entirely understood. In some cases the length of the period seems to be a measure of the time necessary for the germ or virus to reach the particular tissues in the body on which it exerts its harmful effects. Thus, the symptoms of rabies do not develop until the virus reaches the central nervous system, and the long incubation period in this disease represents the time required for the virus to travel from the place where it is introduced by the bite of the mad dog to the spinal cord and brain. In other diseases, as in typhoid fever, there is a very rapid increase of the germs and at the same time a gradually increasing reaction on the part of the body, during the incubation period, and it is only when the germs have become sufficiently numerous and the reaction against them sufficiently great, that the symptoms of illness appear. Doubtless other factors operate to explain the incubation period in different diseases.

The *length* of the incubation period is characteristic in each disease and within certain limits is remarkably constant. In typhoid fever the period is ten to fourteen days, in diphtheria two to four days, in mumps two to three weeks, and in rabies one to three months or more. It is necessary to know the usual length of the incubation period for each of the communicable diseases in order to trace cases back to the source of infection. To find the origin of a new case of typhoid fever, for example, the whereabouts of the patient ten to fourteen days earlier must be determined.

It is important to remember that, in many cases, an infected individual may transmit the disease to others during the incubation period, and since he is still up and about, he may spread the infection to many persons.

Period of illness. A continuous or intermittent *fever* occurs during the period of illness in all germ diseases. The character of the fever is constant in each kind of disease. It is thought that fever, unless it goes too high, may be of benefit in helping the body to resist germs.

Period of convalescence. This is the period of recovery after the symptoms have disappeared. Convalescence may set in, as often happens, for example, in typhoid fever, *despite the fact that virulent germs are still being discharged from the body*, and the convalescent patient may be an important source of infection.

REVIEW QUESTIONS—CHAPTER XXII

1. Define and explain the meaning of the following terms: (*a*) *infection*, (*b*) *contamination*, (*c*) *infectious disease*, (*d*) *communicable disease*, (*e*) *contagious disease*, (*f*) *endemic disease*, (*g*) *epidemic disease*, (*h*) *pandemic disease*.
2. What is one outstanding characteristic of pathogenic bacteria? Name two classes of disease-producing bacteria.
3. Define "opportunists," and explain under what conditions they may produce disease.
4. Give an example of a saprophytic organism which may be classed as an "opportunist." Give examples of "opportunist" infections caused by organisms naturally present on the body surfaces.
5. Define "true pathogens." Explain how they differ from "opportunists." Name some diseases caused by them.
6. State four principal factors which govern the start and course of an infection.
7. Illustrate the importance of (*a*) the path of entrance, and (*b*) the number of germs entering the tissues.
8. Define virulence. On what two properties does virulence depend?

9. Do all disease germs naturally possess the same degree of virulence? Illustrate. Is the virulence of any one strain of germs always the same?
10. Under what circumstances may virulence of an organism be (*a*) decreased? (*b*) increased? Define *attenuated.*
11. Give two facts of practical importance about virulence.
12. What is meant by the *host?* Give a formula showing the relationship between the four factors of infection and explain the meaning of the symbols.
13. Define and illustrate the following terms as they are used in describing infectious diseases: (*a*) *acute,* (*b*) *chronic,* (*c*) *primary,* (*d*) *secondary,* (*e*) *local,* (*f*) *generalized,* (*g*) *pyemia,* (*h*) *bacteremia,* (*i*) *septicemia,* (*j*) *toxemia,* (*k*) *focal,* (*l*) *latent,* (*m*) *specific,* (*n*) *nonspecific,* (*o*) *mixed.*
14. Name three phases in the course of infectious diseases.
15. Define and discuss the nature and importance of the incubation period.
16. What clinical symptom characterizes the period of illness?
17. Under what circumstances may a convalescent patient be a source of infection for other persons?

CHAPTER XXIII

REACTION OF THE BODY TO INFECTION

IN THE preceding chapter we used the formula $D = NV/R$ to express the relationship between the factors that bring about infection. The factor R, that is, the resistance offered by the body tissues to the invasion of microbes, is quite as important as the special properties of the germs themselves in determining the character and severity of an infectious disease. In this and following chapters, the nature of this reaction to infection and the ways in which the human body resists the harmful action of pathogenic organisms, will be discussed. This is the subject matter of *Immunology*.

PRINCIPAL FACTORS IN THE BODY DEFENSE AGAINST INFECTION

Resistance to germ diseases depends upon many different factors. In general there are three lines of defense against infection. These are: (1) *external body defenses*, (2) *nonspecific internal defenses*, which depend upon (*a*) the natural germ-killing power of the blood and other body fluids, and (*b*) the activity of certain body cells, especially the leukocytes, and (3) *specific resistance to the invading microbe* (*action of antibodies*).

External defenses. When the skin is intact, it will prevent the entrance of the great majority of disease germs. The healthy mucous membranes also resist the passage of most germs, though they are more easily penetrated than the skin. Many of the natural secretions of the body help to keep out germs. Thus the eyes are protected by the fluid which bathes them. This is made clear when these secretions dry up, as may happen when the diet is for a long time deficient in Vitamin A. Under these circumstances, the eyes are always infected, and by germs which would be harmless in the normal eye. The high acid content of the stomach secretions sometimes serves to kill germs which would otherwise pass into the intestines and infect them; one is less likely to be infected with typhoid bacilli if they are swallowed immediately *after* a meal. The normal vaginal secretions also inhibit the development of most germs.

Certain mechanical features of the body tend to protect against infection. This is most strikingly shown in the anatomy of the nose and deeper parts of the respiratory tract. The nasal cavities are so constructed that they catch dirt and bacteria as in a trap. The walls of the trachea and bronchi are lined with cells having tiny cilia, which are in continuous motion, sweeping dirt and germs upward toward the mouth (Fig. 70). This must certainly help to protect the lungs from an infection. We learn the value of this arrangement when the cilia are injured, as by a poisonous gas, for then pneumonia almost inevitably follows.

These natural external body defenses are doubtless of value in many instances in protecting against organisms of very low virulence. It must be realized, however, that they are of no avail when the body is attacked by truly virulent germs.

NONSPECIFIC INTERNAL DEFENSES

Bactericidal power of normal blood. If we mix the fresh blood-serum of a normal healthy person with a suspension of one of the common bacteria such as *Escherichia coli,* and then make a count of the number of living bacteria in that suspension as compared with the same suspension before the serum was added, we find that the serum has destroyed a certain number of the organisms. By inoculating germs of low virulence into the peritoneal cavity of animals and watching the changes that take place there, it is possible to show that the normal peritoneal fluid has a similar bactericidal (bacteria-killing) power. This capacity of the normal body fluids to kill germs is feeble, and can have no real value in protecting us against virulent germs. It is probable, however, that bacteria get into the blood stream more often than is commonly thought, through minute injuries in the skin or mucous membrane, chronically inflamed tonsils, etc., and if it were not for this bactericidal power of the blood, infection would be very much more frequent. Some cases of natural, inborn immunity to organisms which are ordinarily very virulent—for example, the immunity of rats to anthrax—seems to be explained by unusually strong bactericidal power of the blood for these germs.

Local reaction to infection. Inflammation. When germs have passed through the body surfaces into the deeper tissues, they usually stimulate a more or less intense local reaction at the site of the infection. In response to the injury caused by the bacteria, or their poisons, the process called *inflammation* sets in. By the term *inflammation* is meant the *local reaction caused by any agent that injures the tissues.* A blow, an irritating chemical substance, or foreign body, or other injurious condition, may bring about an inflammatory reaction. But the pathogenic bacteria are the most common causes of inflammation.

The inflammatory process is defensive. It is an attempt to localize the disease process, to eliminate injurious agents, to neutralize and destroy poisons, and to repair damaged tissues. Hence the inflammatory reaction is an important factor in the body defense against germs.

Types of inflammatory reaction. Exudates. *Nearly always, in inflamed tissues, there is an abnormal accumulation of fluids and of cells from the blood and other tissues.* This material is called the *exudate.* The character of the exudate varies somewhat with the part of the body affected, but it is principally determined by the kind of germ causing the infection. The inflammatory process differs in intensity in different infections, and the exudate differs in amount and in composition.

Special forms of inflammation. In tuberculosis, there is a peculiar reaction resulting in the formation of characteristic *tubercles.* This is so constant a feature of tuberculous infection, that a diagnosis of tuberculosis may be made when tubercles are seen, even though the germs themselves have not been demonstrated.

In a few infections a *serous exudate* forms. This is a clear fluid, containing few cells of any kind.

In diphtheria the exudate takes the form of a tough, grayish white, leather-like membrane, covering the inflamed tonsil, or other part. This is called a *membranous exudate.*

The acute inflammatory reaction. Purulent exudates. The most common result of an *acute* bacterial infection is the formation of an exudate consisting mainly of *polymorphonuclear leukocytes* (the white blood cells with many-shaped nuclei).

These cells are attracted to the infected region and wander there from neighboring blood vessels. When, through the action of proteolytic enzymes from these leukocytes, the exudate becomes liquefied it is called a *purulent exudate* or *pus.* Pus is made up of small amounts of fluid from the blood or lymph vessels, some fibrin and red blood cells, and masses of the polymorphonuclear leukocytes. These latter cells are such prominent constituents of pus that they are commonly called *pus cells* (Fig. 78).

Cellular reactions in subacute or chronic inflammations, where injury to the tissues is relatively slight, are characterized by an accumulation of *lymphocytes,* or other *mononuclear cells,* derived from the blood or from the tissues, rather than by the presence of polymorphonuclear leukocytes. Pathologists thus recognize several varieties of inflammatory cells, besides the polynuclears, which also contribute to the defense of the body against germs.

Phagocytosis. The protective action of acute inflammatory exudates is due principally to the special powers of these polymorphonuclear leukocytes. These cells can engulf (take into their own cytoplasm) foreign particles of all kinds, including disease germs. They circulate in the blood stream, but they have the power of independent motion, (ameboid motion), and they are able to migrate out from the very small blood vessels to a point in the tissues where they are attracted by some particle of foreign material—a group of germs, for example. Once in contact with the germs, the leukocytes take many of them into their own substance just as an ameba surrounds and engulfs a particle of food. When pus is examined under the microscope, many of the organisms are seen to be *within the cytoplasm of the pus cells* (Fig. 78). The engulfed bacteria are sometimes destroyed then and there within the leukocyte, or they may be carried away to their eventual destruction with the death of the cell itself. Metchnikoff, who first called attention to the remarkable action of these leukocytes, named them *phagocytes* (eating cells), and the engulfing of micro-organisms by leukocytes is known as *phagocytosis.*

The power of phagocytosis is not confined to the polymorphonuclear leukocytes, but is possessed in different degrees also by a variety of other body cells, some of which are wandering tissue cells, and some of which are fixed tissue cells. Among the most actively phagocytic of the latter are the endothelial cells lining

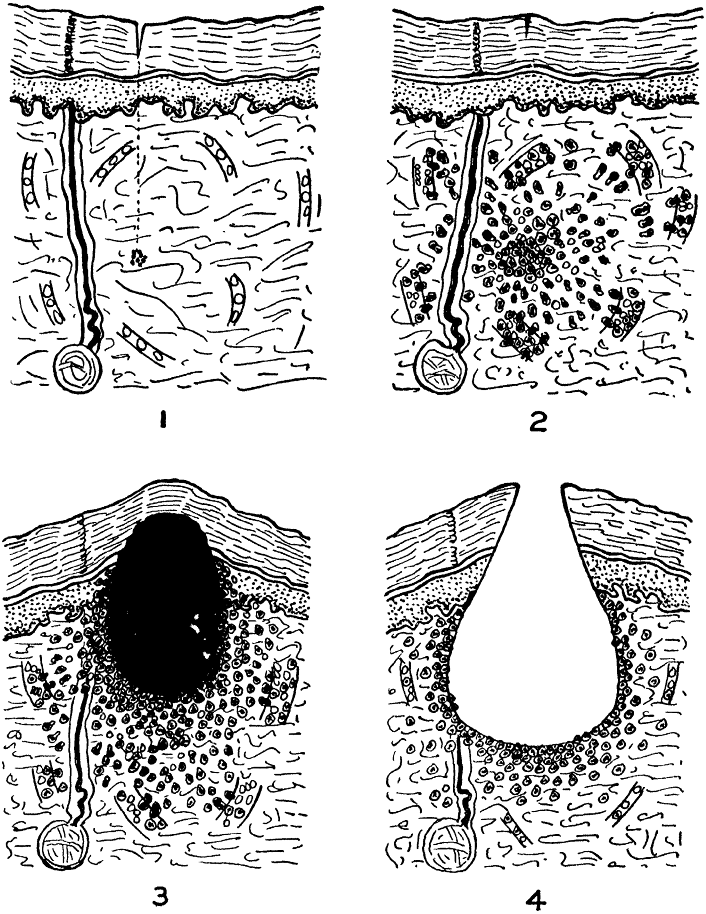

FIG. 59. The "history of a boil." 1. A cut-section of the normal skin just after it has been pierced by a dirty pin, carrying in staphylococci.

2. The early stages of inflammation; migration of leukocytes from the swollen capillaries toward the infected area.

3. The accumulation of leukocytes has continued and now there is a mass of purulent exudate about the germs. The boil has "come to a head" and the semiliquid pus can be seen through the thin cuticle over it.

4. The boil has finally ruptured and the pus has escaped, carrying with it the staphylococci. The migration of leukocytes has ceased and the capillaries are returning to their normal size. New tissue will grow in. (Adapted from Emerson, "Essentials of Medicine." Courtesy of J. B. Lippincott Co.)

the capillaries and sinuses in the liver, spleen, and lymph nodes, and other cells belonging to the so-called reticulo-endothelial system. Most important of the wandering phagocytic cells are those in the group of large mononuclear cells known collectively as *macrophages* to distinguish them from the smaller phagocytes, i.e., the leukocytes. The macrophages are the scavengers that finally clear away cellular débris, bacteria, leukocytes and all, when the infection has been conquered.

Example of an inflammation with pus-formation. History of a boil. The familiar boil, caused by the *Staphylococcus aureus*, is a good example of an acute inflammatory process characterized by the accumulation of pus at the site of infection. Figure 59 illustrates the stages in the development of this inflammation. The process begins a few hours after the staphylococci have entered the deeper layers of the skin and multiplied there. The first stage of the reaction is marked by an enlargement of the capillaries in the region and, therefore, an increased flow of blood through the infected area. The leukocytes, circulating through these distended vessels, are attracted to the spot where the bacteria are growing. This attraction is probably due to chemical substances which diffuse out from the mass of organisms and injured tissue. At first the leukocytes pass more slowly, then they appear to stick on the walls of the blood-vessels opposite the infected part. Finally, the attraction becomes so great that they force their way through the thin walls of the swollen capillaries and travel by ameboid motion toward the germs. Soon there is an accumulation of a great many of these cells in the inflamed tissues, along with some fluid from the blood and surrounding tissues.

After a time, this pus becomes concentrated at one point under the skin, and can be seen as a yellowish "head" protruding above the surface. The skin in the region of the boil now shows all the cardinal signs of inflammation—it is reddened, hot, swollen, and painful. The redness and heat are due to the increased amount of blood there, the swelling is caused by the accumulated exudate, and the pain is due to the pressure of this exudate upon the nerves.

The boil is a typical *abscess*—that is, *a localized accumulation of pus held within a pocket or cavity in the tissues formed by the destruction of the normal tissues.*

In the boil further changes soon occur. Most of the leukocytes die, much of the surrounding tissue is also destroyed, and the pus becomes semiliquid. If the pressure of the accumulated exudate is sufficient, the boil now ruptures, and the pus oozes out, carrying with it a mass of dead tissue, and the staphylococci. The same result is achieved if the head of the boil is lanced at the proper time. Thus the germs are eliminated, and the deeper parts of the body are protected from invasion.

Leukocytosis. The accumulation of pus in any part of the body is usually accompanied by an *increase in the number of leukocytes in the circulating blood.* This is the condition known as *leukocytosis.* There are normally 5,000 to 8,000 white blood cells per cubic millimeter of the blood in a healthy person. About 70 per cent of these are polymorphonuclear leukocytes of the type found in pus. Most acute infections give rise to a marked increase in the number of polymorphonuclear leukocytes, so that these cells may constitute 90 per cent of all the white blood cells, and at the same time there is an increase in the total number of leukocytes present in the circulating blood. In pneumonia, for example, there is a leukocytosis with 20,000 or 30,000 or more white blood cells per cubic millimeter, and a very high proportion of these are of the polymorphonuclear type. Also, an increased percentage of the leukocytes are of the less mature types, indicating that the blood-cell-forming organs are pouring relatively young white blood cells into the circulation to meet the emergency.

Importance of pus-formation and phagocytosis in the body defense against infection. It is obvious, from the story of the boil given above, that the capacity to form pus is of very real value to the body in defending itself against staphylococci. It happens that these organisms have an especially marked tendency to form localized abscesses similar to a boil in any part of the body they may invade, and pus-formation is so characteristic a reaction to staphylococcus infection that these germs are commonly called *pyogenic cocci* (pus-generating cocci). A few other germs are also markedly pyogenic. In pneumonia, gonorrhea, and meningitis, for example, most of the symptoms are really due to the accumulation of pus in the infected tissues, and at the same time the phagocytic action of the pus cells is an important factor in the body defense.

In many other infections actual pus formation does not occur, although more or less sizable accumulations of inflammatory cells appear in infected areas. One fundamentally important effect of this cellular reaction is to help *localize* the infection, and reduce the liability of a more serious generalized invasion of the body.

Metchnikoff believed that the leukocytes and other phagocytic cells were responsible for the entire phenomena of immunity to germs. It would be difficult indeed to overstate their importance. It is always the phagocytes, particularly the macrophages, that finally dispose of invading bacteria. But in most virus infections, in diseases like diphtheria where the body is poisoned by a toxin, in spirochetal infections such as syphilis, and in other common germ diseases, local cellular reactions are slight, and phagocytosis does *not* play an important part in defense. Moreover, even in the frankly pyogenic infections where phagocytosis is most active, the leukocytes do not act entirely alone, for the bacteria are first made susceptible to the phagocytes by antibodies called *opsonins* present in the blood serum and other body fluids. Finally, there is nothing specific about the action of phagocytes, since they may take up several different kinds of disease germs with equal readiness, and their activities do not result in any permanent immunity. It follows that the specific and lasting resistance many persons have toward certain particular germs cannot be dependent on phagocytosis.

DEVELOPMENT OF SPECIFIC RESISTANCE. FORMATION OF ANTIBODIES

Recovery from most germ diseases is accompanied by deep-seated physiological changes in the whole body, as a result of which the individual becomes resistant to the particular organism causing the illness. No one knows exactly what constitutes these changes, but it is clear that some reaction goes on between the invading germ and the tissues of the infected individual during the course of the disease, so that the individual, at first susceptible, becomes, at the time of recovery, highly resistant toward that particular germ. Everyone is familiar with the fact that this newly acquired immunity often persists for years, and that a person who has recovered from typhoid fever, measles, and other common infections, is not likely to have the same disease again.

The physiological processes which underlie immunity are exceedingly complex and by no means entirely understood. It has been found, however, that the development of a specific resistance toward a certain disease germ is associated with the appearance in the blood of so-called *antibodies.* These antibodies are *capable of reacting specifically with the particular organism, or toxin, which caused their formation.* Thus, a person infected with typhoid bacilli develops in his blood antibodies which act upon these germs, and a person poisoned by the toxin of the diphtheria bacillus develops an antibody which acts upon this toxin, and so on.

Before discussing the nature and origin of these antibodies, it will be helpful to get a clear idea of what they do.

Principal kinds of antibodies and their action. The principal antibodies which are formed as the result of contact with bacteria, or their toxins, are of two general kinds: (1) *antitoxins,* and (2) *antimicrobial antibodies.* Antitoxins are developed by the body *only in response to the presence in the tissues of an exotoxin, i.e.,* the type of toxin produced by the diphtheria bacillus and a few other germs (p. 135). Antitoxins *neutralize* these toxins and make them harmless. Antimicrobial antibodies form whenever the body is infected or artificially vaccinated with microbes. They react in some way *upon the micro-organisms themselves.* Among these antibodies are included the kinds called (*a*) *bacteriolysins,* (*b*) *agglutinins,* (*c*) *precipitins,* and (*d*) *opsonins.*

The drawings in Figures 60 and 61 show diagrammatically the action of the principal antibodies and how this action is tested for in the laboratory.

Antitoxins. The kind of antibody called antitoxin was first demonstrated by Behring and Kitasato in connection with their early studies of resistance to tetanus and diphtheria (1890). They found that animals which had survived a series of injections of small amounts of tetanus toxin had become very resistant to this poison. In attempting to find an explanation of this immunity, they were naturally led to examine the blood of the resistant animals. Since whole blood could not be studied conveniently, they gave their attention to the blood *serum*—the clear, yellowish fluid which separates from clotted blood. They found that *the blood serum of animals immune to tetanus contained something which neutralized the poisonous action of tetanus toxin.* The substance or element in the serum responsible for this action they called *tetanus antitoxin.*

When normal (susceptible) animals were injected with some of the serum of these immune animals (*i.e.*, with serum containing antitoxin), *the injected animals were thereby rendered immune.* The action of this serum from tetanus immune animals was *specific*, that is, tetanus antitoxin protected against tetanus toxin only, and not against diphtheria toxin or other poisons.

Behring showed that resistance to diphtheria, as well as to tetanus, depends upon an antitoxin—in this case *diphtheria antitoxin,* which specifically neutralizes the *diphtheria* exotoxin, the poison secreted by the diphtheria bacillus.

Immunity to diphtheria or to tetanus is explained very well by the action of these antitoxins (Fig. 60). When antitoxin is present in the blood stream, it combines immediately with any toxin it may encounter. This combination makes the toxin harmless and, therefore, prevents it from injuring the tissues. Tetanus in human beings may be *prevented* by the injection of a serum containing tetanus antitoxin, and diphtheria may be *cured,* as well as prevented, by injection of a serum containing diphtheria antitoxin.

A considerable number of poisons of animal or plant origin, such as these of the scorpion and the spider, snake venom, and ricin (a poisonous substance in the castor oil bean), are capable of causing the production of specific antitoxins when injected into animals. The fatal poisoning that follows the bite of certain snakes can be prevented by the injection of a serum containing antitoxins (antivenins) which will neutralize the deadly venom of these snakes. The exotoxins formed by *Streptococcus scarlatinae, Clostridium welchii, Clostridium botulinum,* and a few other disease germs, all stimulate the body to form specific neutralizing antitoxins.

Antimicrobial antibodies. The *endotoxin* contained in the bodies of nearly all pathogenic organisms, however, *does not cause the formation of antitoxin.* Hence, no antitoxin develops in persons with typhoid fever, for example. *Antitoxin plays no part in the body defense against the great majority of germs.* Diphtheria,

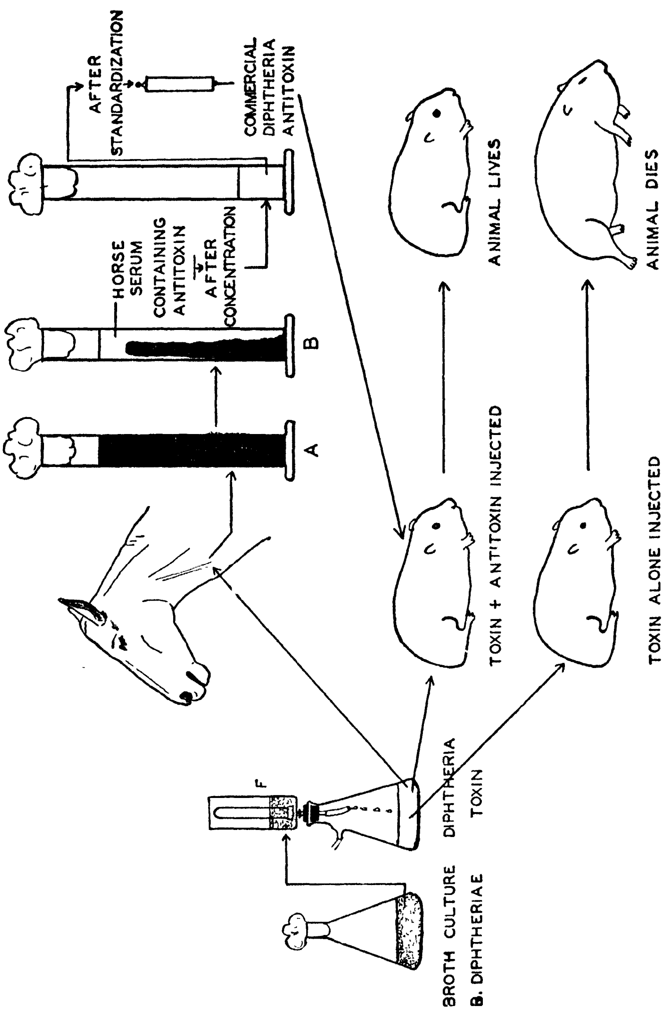

FIG. 60. (Legend is on opposite page.)

scarlet fever, and tetanus are the most common human diseases which are caused by exotoxin-producing organisms, and which may be successfully treated or prevented by an antitoxin. In these few diseases the action of antitoxin seems to explain immunity, but antibodies of a different sort must be concerned with resistance to typhoid bacilli, cholera spirilla, and the many other pathogenic organisms that produce little or no exotoxin. These antibodies must be of the kind that act upon the germ itself, i.e., antimicrobial antibodies.

Bacteriolysins. The experiments of Pfeiffer (1894) were among the first to demonstrate the existence of antibodies of this kind. Pfeiffer found that, when virulent cholera spirilla were inoculated into the peritoneal cavity of guinea pigs immune to this organism, the spirilla rapidly became swollen, then granular, and finally disappeared altogether. In other words, he showed that the peritoneal fluid of immune animals had the power to destroy cholera spirilla. He further demonstrated that this power was possessed to a very limited extent only by normal animals, but was developed to high degree during the course of a cholera infection, or as a result of a series of injections of the spirilla. The destructive action of the peritoneal fluid of the immune animal was evidently due to a kind of "immune substance"—or, as we say now, an *antibody*, developed by the animal in response to the presence of the germs. This antibody is called a *bacteriolysin*, meaning a substance which causes the lysis (dissolution, destruction) of bacterial cells.

Metchnikoff, Bordet, and others soon showed that the bacteriolytic power of the body fluids, including the blood, of animals immune to cholera and other germs, can be demonstrated in test tube laboratory experiments as well as in the animal body

FIG. 60. Diagram illustrating the production and action of antitoxin, using diphtheria antitoxin as an example. At the left is shown how diphtheria *toxin* is secured. After *C. diphtheriae* has grown in broth for several days the culture is passed through a filter (F), and *the clear, sterile filtrate constitutes diphtheria toxin.* When this is injected repeatedly into the horse, the animal responds by the formation of a specific *antitoxin,* and this becomes abundant in the blood of the animal. To secure the antitoxin the horse is bled, and from the blood (A), after it has been allowed to clot (B), the clear blood *serum* is separated. This serum, after concentration, purification, and standardization is put upon the market as diphtheria antitoxin. The action of this antitoxin in protecting against the diphtheria toxin is demonstrated by animal tests, as illustrated.

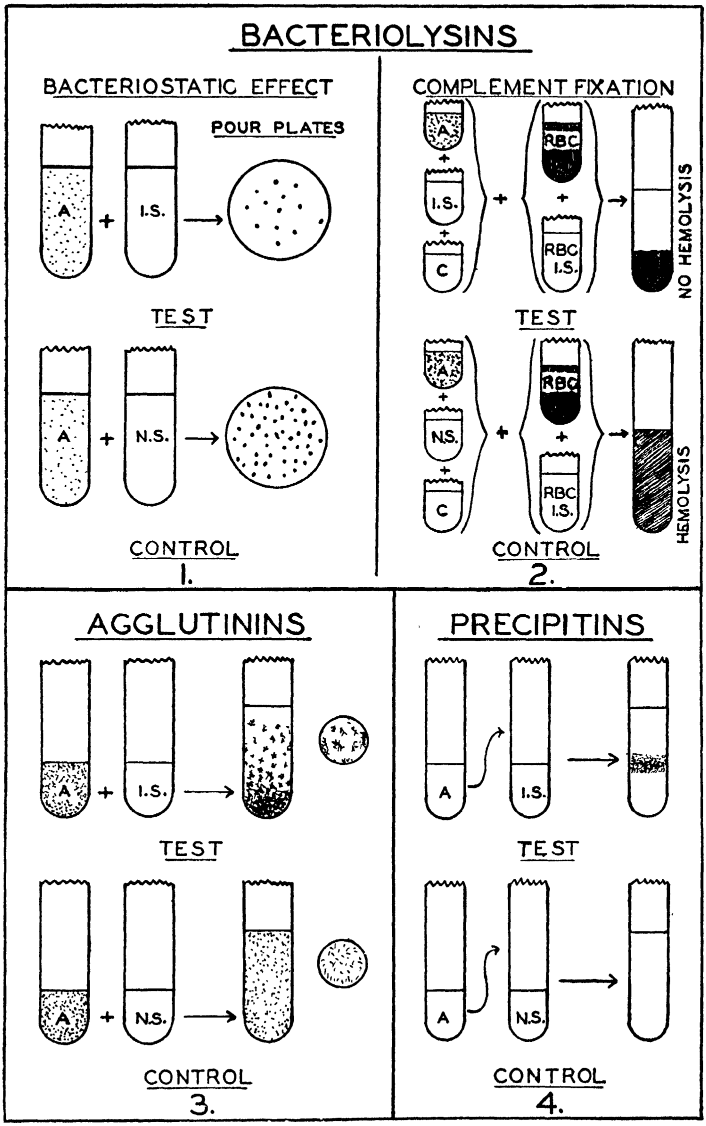

FIG. 61. (Legend is on opposite page.)

(Fig. 61, 1). They found that bacteriolysis depends upon the interaction of two elements with the bacterium: (1) *the specific antibody* (bacteriolysin), and (2) *an unstable element normally present in the blood* and other body fluids of human beings and animals, which is now called *complement.* The bacteriolysin is produced in large amount when an individual becomes immune. Its action is *specific*, that is, it can combine only with the kind of bacterium which caused its formation. Complement, on the other hand, is naturally present in blood and other body fluids of normal individuals and is not increased in amount by immunization. Complement is the active element in causing bacteriolysis, but it can do so only after the antibody (bacteriolysin)

Fig. 61.—Diagrams illustrating the action of bacteriolysins, agglutinins, and precipitins.

Diagram 1. When a suspension of bacteria (A) is mixed with the *fresh* blood serum of an animal immune to the organisms, *i.e.*, unheated immune serum (I.S.), and agar plates are then poured from the mixture, the resulting growth shows that many of the organisms in A have been killed, or at least, prevented from growing (bacteriostatic effect), since in this plate there are fewer colonies than in the control plate made from the same suspension mixed with normal serum (N.S.). This effect of the immune serum is due to the antibody called *bacteriolysin.* The action of the bacteriolysin, however, depends upon the presence of *complement* in the serum. This is destroyed by heat, hence the serum must be fresh and unheated.

Diagram 2. When a suspension of bacteria (A) is mixed with its specific immune serum (I.S.) which has been heated to remove its natural content of complement, and then a measured quantity of complement (C), (in the form of fresh guinea pig serum) is added, the bacteriolysin in the immune serum combines with the organisms and *also with the complement,* so that the complement becomes bound, or "fixed." To demonstrate this fixation of the complement a mixture of a red blood cell suspension (R.B.C.), and an immune serum specific for red blood cells (R.B.C.I.S.) is added. Hemolysis of the red blood cells, for which complement is necessary, does not occur, because there is no free complement there. In the control, on the contrary, using normal serum (N.S.), the complement is not fixed, and so is free to act when the red blood cell mixture is added, and hemolysis of the cells occurs.

Diagram 3. When a specific immune serum (I.S.), is added to a suspension of a bacteria (A), the organisms clump together into visible masses which may fall out of suspension. This phenomenon of agglutination is due to an antibody called *agglutinin.* Normal serum (N.S.) has no effect. At the right the microscopic appearance of the mixtures is illustrated.

Diagram 4. When a clear solution of a certain bacterium or other protein matter, is added to the serum of an animal immunized to that particular bacterium, or protein (I.S.), a precipitate occurs. This is due to the antibody called *precipitin.* The reaction is most striking when the mixture of protein solution and immune serum is made in such a way that one liquid is stratifie over the other, the precipitate forming at the junction of the two. Norr serum (N.S.) has no effect.

jehas combined with the bacterial cell and apparently prepared it for the action of the complement.

It is generally assumed that bacteriolysins play an important part in resistance to many germ diseases.

Agglutinins. Shortly after the discovery of bacteriolysins, Gruber and Durham called attention to another antibody reaction. They noticed that when suspensions of certain bacteria were mixed in a test tube with the serum of animals immunized against these bacteria, the organisms clumped together into visible masses which finally settled to the bottom of the tube. The same phenomenon of clumping of the organisms could be seen in drops of a mixture of serum and bacteria under the microscope (Fig. 61, 3). They spoke of the clumping as *agglutination;* the organisms were said to be *agglutinated.* The specific antibody in the serum responsible for this phenomenon was named *agglutinin.*

Agglutinated bacteria are not necessarily killed, but undoubtedly the clumps of organisms are more likely to fall prey to phagocytes.

Precipitins. In 1897 Kraus described the most striking of all test tube antibody reactions. He found that, when a clear solution or extract of certain bacterial cells, or any clear fluid containing some of the protein matter from those bacteria, is mixed with clear serum from animals immune to these organisms, a *precipitate* occurs (Fig. 61, 4). This reaction was attributed to an antibody appropriately called a *precipitin.*

Opsonins. We have previously mentioned that the engulfing of microbes by leukocytes (phagocytosis) is not accomplished by the unaided efforts of the phagocytes alone, but occurs in the presence of blood or other body fluids. The importance of these fluids in furthering phagocytosis was noticed as early as 1895 by Denys and LeClef, but was first studied extensively by Wright and his associates beginning in 1903. These investigators showed that the blood serum of normal men and animals when added to a mixture of washed leukocytes and bacteria had a slight effect in aiding the engulfing of the organisms by the leucocytes, but that the blood serum from an *immune* individual had this phagocytosis-promoting property to a far greater degree. was shown that this effect of an immune serum is specific, and action is upon the bacteria, preparing them for phagocytosis, rather than directly upon the leukocytes. Wright named the phagocyte-helping substances in the serum "*opsonins*," a word derived from the Greek meaning "to prepare food for, to cater to." Opsonins then, are antibodies that prepare or sensitize bacterial cells so that they are made especially susceptible to phagocytosis.

The importance of these antibodies in defense when the tissues are invaded by virulent bacteria is obvious. The opsonins assist the phagocytes in the necessary process of capturing and removing the harmful microbes.

Nature of antibodies. It will be clear from this brief account that *antibodies are named according to their action.* They have not been separated in strictly pure state from the blood serum of immune individuals where they occur. There is no way of proving the presence of antibodies in such a serum or of determining their kind or amount by ordinary physical or chemical tests. The antibodies can be demonstrated only by bringing the serum containing them into contact with the particular germ or toxin which caused their formation and with which they will react in one of the ways described above. We know antibodies, therefore, by what they do, and we name them accordingly.

It must not be imagined that the antibodies differently named are necessarily different substances. It is now generally agreed that the different phenomena of bacteriolysis, agglutination, and so on, are actually not due to different, distinct substances in the serum of the immune individual as was originally thought, and as the names bacteriolysin, agglutinin, and so forth, would suggest. Rather, the power of an immune serum to cause specific bacteriolysis, agglutination, and the other reactions with a particular organism, is due to a single antibody. Which one of these reactions is observed in a particular test or experiment depends upon the conditions under which the serum and the organism are brought together.

Antibodies probably consist actually of *serum globulins, altered from their normal physico-chemical makeup in a specific way because they were formed by the body cells in the presence of a particular foreign substance.* Their specialized structure makes them capable of combining specifically with that substance.

Antigens. It is important to realize that *the body forms spe-*

cific antibodies not only when it is invaded by bacteria or poisoned by their toxins, but also *whenever foreign protein material of any kind is introduced into the tissues.* Antibody formation is, then, a general biological phenomenon. It is one of the ways living tissues respond to the presence of foreign matter. *It happens that, as a general rule, the antibodies formed when we are infected, or artificially vaccinated, with microbes, help to protect the body against the germs or their toxins, and these antibodies are to a large extent responsible for our resistance to infectious diseases.*

Any substance which, when introduced into the tissues, will stimulate the formation of antibodies, is called an *antigen*, or, more properly, a *complete antigen.* Practically all *proteins* are complete antigens. Bacterial cells and toxins, of course, contain much protein. Carbohydrates and other nonprotein materials usually are not able to stimulate antibody formation when injected by themselves, though when combined with protein they sometimes determine the specific character of the antibody which the whole compound produces. They also may react with this antibody in the test tube and in the animal body. Such substances are called *partial antigens*, or *haptens.* An example of a hapten is the carbohydrate present in the capsule of a *Pneumococcus*, which determines its "type" (see Chapter XXXII).

Measurement of the antibody content of a serum. The phenomena of agglutination and other antigen-antibody reactions described above may be employed not merely to demonstrate an antibody of a particular kind in a sample of blood serum, but also to learn how much of the antibody is present. This measurement is important especially for the purpose of standardizing serums, containing antitoxins or antibacterial antibodies, which are intended to be used in the treatment of disease.

The amount of *antitoxin* in a serum is determined by inoculating animals with the serum after mixture with different amounts of the corresponding toxin, thus testing the capacity of the antitoxin to protect animals against the poison. The protective power of the serum is expressed in terms of the antitoxin *units* it is found to contain. (See Chapters XXVIII and XXX.)

The amount of *agglutinin* in a serum is measured by making a series of dilutions of the serum with physiological salt solution and testing each dilution for its agglutinating power. The highest dilution at which agglutination of the bacterial suspension takes place is said to be the *titer* of the serum. Thus, if agglutination still occurs when the serum is diluted up to 1:5,000, but not at higher dilutions, the serum is said to have an agglutination titer of 1:5,000. By the artificial immunization of animals it is often possible to secure an agglutinating serum which will be active in a dilution as high as 1:30,000 or more. The *precipitin* content of a serum is measured in a similar manner. Precipitins are active in very high dilution.

The *opsonic power* of an immune serum may be estimated by mixing a measured amount of the serum with leukocytes and with a dilute suspension of staphylococci (or other bacteria). The amount of phagocytosis in this mixture is compared with the amount in a similar mixture prepared in the same way but containing only sterile *normal* serum. Smears of the mixtures are made after they have been incubated for a time, and by counting the number of bacteria engulfed by 100 leukocytes in each mixture the phagocytic activity may be expressed numerically, and the relative opsonic power of the immune serum over that of the normal serum may be stated in a figure called the *opsonic index.* The practical value of opsonic index determinations is very limited, however, because of the technical difficulty of the tests, and unless performed by experts in special circumstances little significance can be attached to them.

Protection tests. Test tube reactions alone will not reveal all the special properties of antimicrobial serums. Most significant, of course, is the ability of such a serum actually to protect a susceptible animal against infection. The sterile serum and the active bacterium or virus concerned are mixed, the mixture is allowed to incubate for a short time, then it is inoculated into fully susceptible animals of an appropriate species. Specific protective action is indicated when animals receiving such mixtures survive, while control animals, injected with the same material without serum, or the same organism with a serum of different origin, succumb to the infection.

The wide use of such protection tests to determine the identity of viruses has previously been mentioned (p. 94). Such tests are used, too, to measure the potential value of antipneumococcus serum and other serums intended for treatment of human disease. Often no direct correlation is found between the concentration of antibody in a particular serum as shown by test tube reactions, and its protective power. This means that the blood of immune individuals may possess defensive powers beyond detection by artificial test tube procedures.

PRACTICAL USE OF ANTIGEN-ANTIBODY REACTIONS

Diagnostic agglutination and precipitin tests. Tests for agglutinins, precipitins, and other antibodies *in the patient's serum* are of immense value in the *diagnosis of disease.*

Agglutination tests. One of the simplest methods for the diagnosis of typhoid fever is the so-called Widal test. In this test we put some of the patient's serum in contact with a suspension of *known* typhoid bacilli. If the bacilli are agglutinated (as can be seen in a test tube or under the microscope) the *patient's serum must contain antibodies (agglutinins) for these organisms.* Since antibodies are specific and react only with the germs which caused their formation, this means that the individual has been infected, or vaccinated, with typhoid bacilli sometime previously, or else he is now infected with them. Of course, if he has symptoms of typhoid fever, the latter is probably the case. If we make the same test a day or two later, and repeat at intervals, and find that the typhoid antibodies are increasing in amount in the serum, this is positive proof that the case is one of typhoid fever, because the only way in which a person could manufacture these particular antibodies is by being infected (or vaccinated) with this particular antigen (typhoid bacillus).

Similarly in other diseases a diagnosis can often be made by demonstrating agglutinins for the germ in the patient's blood, even though the germs themselves cannot be isolated or identified. Among the infections in which laboratory diagnosis is commonly made chiefly on the basis of such agglutination tests are, (besides typhoid fever), bacillary dysentery, brucellosis (undulant fever), tularemia, and typhus fever.

Precipitin tests. The technique of precipitin reactions is not widely used in routine diagnostic work, but occasionally precipitin tests are performed as part of the laboratory examination of spinal fluid, blood, sputum, urine, or other materials obtained from the patient. In pneumonia, for example, the *type of pneu-*

mococcus present in the sputum may be determined by precipitin tests.

Diagnostic complement fixation tests. As we have already explained, complement is not itself an antibody, but a normal constituent of fresh blood serum. Nevertheless it takes an essential part in certain antigen-antibody reactions. Complement is the active element which brings about *bacteriolysis* of cholera spirilla, for example, in the serum of animals immune to cholera, and in an exactly similar way it is the agent that causes disintegration of red blood cells (*hemolysis*) in the serum of an animal immunized to these cells. But in either case, the complement acts only after the antigen (bacterial cells or red blood cells) has been "sensitized" by combination with its specific antibody (*bacteriolysin* or *hemolysin*). The complement becomes absorbed, or *fixed*, upon this antigen-antibody complex. The antibody appears to combine with *both* its specific antigen and with complement, and when this combination is complete, bacteriolysis (or hemolysis) takes place. Ehrlich called an antibody of this kind an *amboceptor* (from Latin, *ambo*, both, and *capere*, to take).

Thus,

bacterial cells + bacteriolytic amboceptor + complement = bacteriolysis
(antigen) (antibody)
red blood cells + hemolytic amboceptor + complement = hemolysis
(antigen) (antibody)

For diagnostic purposes complement-fixation tests are designed to demonstrate whether or not the patient's serum contains an amboceptor (antibody) specific for a certain disease-producing organism. To make the test a suspension of these organisms (antigen) is mixed in suitable proportions in a test tube with the patient's serum (which may or may not contain the antibody being tested for), and with complement (usually supplied by fresh guinea pig serum) (Fig. 61, 2). This mixture is incubated at 37° C. for an hour. During this time, if the patient's serum contains the amboceptor specific for the germs, combination occurs, and as a result the complement is absorbed or fixed. However, if the patient's serum lacks the specific amboceptor there will be no combination, and the complement will remain free. There is no visible change in the appearance of the mixture in the test tube to show what may have taken place. The next step, then, is to add to the mixture something which will indicate whether or not free complement is present. The indicator used is a suspension of red blood cells plus their specific hemolytic amboceptor. Measured amounts of this suspension are added and the mixture incubated again. Now, if complement is free in the mixture, it will cause hemolysis of the added red blood cells, which produces an easily visible change in the appearance of the mixture. If, on the contrary, all the complement was fixed during the first incubation so that none is now available, the red blood cells will remain unchanged. *Complete hemolysis* means *a negative reaction, i.e.*, the antibody tested for is not present in the patient's serum. *No hemolysis* means a *strongly positive reaction* (usually reported as four plus), *i.e.*, sufficient antibody present in the patient's serum to fix all the complement. *Partial hemolysis* means a *weakly positive reaction, i.e.*, some antibody present in the patient's serum but not enough to absorb all the complement.

The *Wassermann test for syphilis* is a complement-fixation test. The antigen preparation used is not a suspension of the causative germ, however, but a colloidal solution containing lipoids extracted from muscle, which has been found to be superior as an antigen for detection of the particular antibody formed by the syphilitic patient.

Skin tests. Another type of antigen-antibody reaction is that produced when a minute amount of a preparation containing the bacterium or virus (antigen) is introduced into the skin of a person or animal who is infected, or has been infected, with that organism. A local reaction will occur if antibodies for the injected antigen are present in the tissues. Such *skin tests* are useful in diagnosis, in some instances, for example in tuberculosis (*tuberculin test*) and in lymphogranuloma inguinale (*Frei test*).

Identification of unknown organisms and proteins. Antigen-antibody reactions are valuable not only for demonstrating presence of a particular antibody in a patient's blood, but also as a means of *identifying unknown organisms.* If, for example, a culture is isolated from a case of typhoid fever and is suspected to be typhoid bacilli, the identity of the organisms can be finally established by mixing a suspension of them with a serum *known* *to contain typhoid antibodies.* If agglutination occurs, under properly controlled conditions, the germs must be typhoid bacilli.

Precipitin reactions are especially useful in identifying unknown protein materials. For, example, by using precipitin tests it is easy to determine whether a blood spot is of human or animal origin. It is only necessary to have in the laboratory a serum *known to contain precipitins for human blood.* If a solution of the unknown blood gives a precipitate when mixed with this serum, and not in control preparations, it must be human blood. Such tests (referred to as *forensic precipitin tests*) are widely employed in medico-legal work.

REVIEW QUESTIONS—CHAPTER XXIII

1. What are the three lines of defense against infection?
2. Discuss the external defense of the body against infection.
3. What importance has the bactericidal power of normal blood?
4. Define *inflammation.* What is the purpose of the inflammatory process?
5. What germ causes the formation of tubercles? Define *exudate.* Give examples of infections resulting in (*a*) a serous exudate, (*b*) a membranous exudate.
6. What is the usual character of the acute inflammatory reaction? How does the cellular reaction differ in less acute inflammations?
7. What is meant by *purulent exudate, pus, pus cell, polymorphonuclear leukocyte, phagocyte, macrophage, phagocytosis?*
8. Describe phagocytosis. What types of body cells have phagocytic power?
9. Trace the history of a boil. Define *abscess.*
10. What is meant by *leukocytosis.*
11. What is meant by *pyogenic* organisms? What importance has pus-formation and phagocytosis in defense against infection?
12. What explains recovery from most germ diseases and specific resistance against particular organisms?
13. Explain as clearly as possible what you understand by the term *antibody.*
14. Name the principal kinds of antibodies. Under what conditions are *antitoxins* developed?
15. Discuss the discovery, production, and action of antitoxins.
16. Name three common human diseases which may be successfully prevented or treated by an antitoxin.
17. Discuss the discovery and action of bacteriolysins.
18. Discuss the discovery, and action of (*a*) agglutinins, (*b*) precipitins, (*c*) opsonins.

19. What is known about the nature of antibodies?
20. Discuss antibody formation as a general biological phenomenon and explain its particular significance in relation to immunity.
21. Define *antigen, complete antigen, partial antigen, hapten.* Give examples.
22. Discuss briefly methods of determining the amount of antitoxin, agglutinins, precipitins and opsonins in a serum. What are protection tests and how are they used?
23. Explain how antigen-antibody reactions are useful in (*a*) diagnosis of disease, (*b*) identification of unknown bacteria and protein substances.
24. What is meant by forensic precipitin tests? What is the principle of skin tests, such as the tuberculin test?
25. Explain the principle of complement-fixation tests. In what way does the Wassermann test differ from other complement fixation tests?

CHAPTER XXIV

NATURAL AND ACQUIRED IMMUNITY

IN THE previous chapter we have described some of the important reactions of the body which are associated with resistance to infection. We shall now consider immunity in a wider sense. Under what circumstances are human beings immune to our common germ diseases? How can this immunity be purposely acquired? It is important to understand this very practical aspect of the subject, because artificial methods of conferring immunity are now widely depended upon to prevent disease.

Immunity. The word *immune* is unfortunately used in two senses. It is sometimes employed to mean *not susceptible.* Practically the only instance, however, in which the term can be properly used in this absolute sense is in the case of the immunity which depends upon differences in species. Thus, human beings are not susceptible (*i.e.,* they are absolutely immune) to hog cholera, because they are humans and not hogs. *But absolute immunity is unknown against any infection to which the species is naturally susceptible.* When we say an individual is immune, we usually mean merely that he has a *relatively increased resistance toward some particular germ.* He is simply more resistant, that is to say, less susceptible, than normal, nonimmune, fully susceptible individuals.

For convenience in discussing this subject the outline of immunity given below will be followed.

AN OUTLINE OF IMMUNITY

I. *Natural Immunity* (immunity at birth).

  (*a*) Acquired from mother before birth (congenital immunity).

  (*b*) Inherited

        (1) *Species immunity* (differences in susceptibility of various species).
        (2) *Racial and group immunity* (differences in susceptibility of different races and groups of the same species).
        (3) *Individual immunity* (differences in susceptibility of different individuals of the same race).

II. *Acquired Immunity* (immunity acquired during life).
    (*a*) By recovering from the disease, or by carrying the germs without being ill.
    (*b*) Artificially, by use of
        (1) *vaccines* (active immunization).
        (2) *immune serums* (passive immunization).

Natural immunity. What is called *natural immunity* depends upon factors which are *inborn,* and part of the heritage of the race to which an individual belongs. This must be distinguished from *acquired immunity,* by which is meant the immunity developed as the result of contact with germs during the life of an individual.

Congenital immunity from the mother. For a few months after birth many infants show a resistance toward certain diseases because of antibodies acquired from the mother during uterine life. For example, babies often have considerable amounts of diphtheria antitoxin in their blood passed to them from the blood of their mothers, who were immune to diphtheria. Similarly, infants may possess a specific resistance to other diseases to which the mother is immune. This form of natural immunity is not of great practical importance, however. It usually disappears by the end of the first year of life.

Inherited natural immunity. Apart from this transient immunity acquired from the mother, individuals inherit as part of their constitution, in common with other members of their race and species, varying degrees of natural resistance toward infection. If we take any particular disease, we find that there are marked differences in susceptibility to that disease in certain species of animals as compared with other animal species, or with human beings, and also there are differences in susceptibility among different races and groups of the same species, and among the individuals of the same race.

(1) *Species immunity* (differences in susceptibility of various species). In general, the cold-blooded animals (fish, frogs, turtles, etc.) are not susceptible to diseases common to warm-blooded animals. The body temperature seems to be one factor in this resistance. For example, the temperature of fowls is higher than that of human beings (about 42° C.), and they are normally resistant to the organisms of plague and anthrax. But Pasteur showed, in one of his dramatic experiments, that a hen chilled by immersion in water until the body temperature is lowered becomes susceptible to anthrax. Differences in the body temperature, however, can hardly account for natural species immunity in all cases. There must be other factors not as yet fully understood.

The differences in susceptibility of different species to tuberculosis germs is especially interesting. Fish have a form of tuberculosis, but the tubercle bacilli which cause the disease in fish will not infect human beings, nor will the human tubercle germs infect fish. Hens and other birds have a spontaneous disease very much like tuberculosis in man, but the avian tubercle bacilli probably never infect human beings. Man is susceptible to the type of tubercle bacilli which infect cattle (bovine type), but much less so than to the human type of the germ. Both rabbits and guinea pigs are very susceptible to the bovine type, but rabbits are much more resistant than guinea pigs to the human type.

Human beings are not susceptible to some diseases of lower animals, such as chicken cholera. There are a number of diseases, however, which are primarily animal infections, but which may be transmitted to man. Among the most important are *anthrax* (cattle, sheep, horses), *glanders* (horses), *plague* (rodents), *Malta fever* (goats), *bovine tuberculosis* (cattle), *tularemia* (rabbits), *rabies* (dogs, cats).

There are many diseases of human beings that do not naturally occur in animals. Examples are *syphilis*, *gonorrhea*, *cholera*, *typhoid fever*, *influenza*, *measles*, *mumps*, and *poliomyelitis*. In the case of some of these diseases, the artificial inoculation of the germs into certain animals will cause development of the disease, but usually the animals are much more resistant to the infection than human beings are. It is an interesting fact that animals closely related to man in the evolutionary scale—chimpanzees and other anthropoid apes—are more likely to be susceptible to human diseases than other animal species. It has been found possible to reproduce experimentally only in these animals such characteristic human diseases as measles, typhoid fever, and poliomyelitis.

(2) *Racial and group immunity* (differences in susceptibility of different races and groups of the same species). Among animals there are several examples of what appears to be a racial immunity. Thus the field mouse is very susceptible to glanders, but the white mouse is immune. Algerian sheep are resistant to anthrax, while common sheep are susceptible.

Among human races there are also differences in susceptibility to common infections, but these are not so marked. Negroes are more resistant to some skin diseases than white people, but they appear to be considerably more susceptible to venereal diseases, pneumonia, and tuberculosis. It may be that differences in mode of life, in hygienic conditions in the homes, and in opportunities for infection explain to a large extent these apparent differences in susceptibility.

A race of people that have been exposed to a disease continuously for a very long time is likely to have a considerable degree of natural immunity to that disease, because only the more resistant stock has survived, and a kind of balance has been effected between the virulence of the germs and the resistance of their hosts. This probably explains why the Caucasian race in general, and Jewish people in particular, are more resistant to tuberculosis, for example, than Negroes and Indians.

When an infectious disease is first introduced into an aboriginal race, it takes a very heavy toll—witness the havoc wrought by tuberculosis and syphilis among Eskimos and American Indians. In the course of time, however, the disease tends to become less severe and it may eventually reach the same level of incidence and severity as it has among people of other races, with whom the disease has been endemic for long periods. When Captain Cook, on one of his famous voyages, touched at the South Sea Islands, the natives contracted measles from a member of his crew. Apparently this disease had never occurred there before. It spread very rapidly, causing severe illness, and killed a large proportion of the native population. Now, however, measles is no more severe in the South Sea Islands than elsewhere. The people there now inherit a degree of natural resistance to measles which the aborigenes did not have.

The group of people inhabiting any particular region show a certain degree of natural immunity to diseases that are common there. This *group immunity* is characteristic of the people as a whole, without regard to race. If we count all human beings in civilized countries in one group, we can say that this group has a considerable degree of natural resistance to many common infections, such as smallpox, syphilis, and tuberculosis. Furthermore, this immunity is always tending to increase. All the diseases just mentioned were more severe one hundred years or more ago than they are now. During this time, many of the more susceptible individuals have been eliminated, and also the most virulent of the germs have died out with their hosts, leaving at the present time a population of relatively resistant individuals and less virulent strains of the organisms. In other words, mankind as a whole is always tending toward a state of balance with the microbes which assail us. Among the people in any one community—comprising a local group—this *man-microbe balance* under normal conditions keeps the communicable diseases at a certain more or less constant level of frequency and severity in that community.

(3) *Individual inherited immunity* (differences in susceptibility of individuals of the same race). Apart from any specific immunity toward any particular germ that they may happen to possess, individuals vary in their capacity to resist our common germ diseases. Some individuals seem to be naturally more susceptible than others to skin disorders, common colds, and other familiar diseases. It is also a well known fact that the natural resistance of the same individual varies from time to time.

These differences cannot be fully explained, but some of the factors which influence natural resistance are easily understood. As pointed out in the previous chapter, many of the mechanical features of the body, for example, the anatomical structure of the nose and deeper respiratory tract, help to keep out germs; and obviously, physical imperfections, such as a deviated nasal septum, flat chest, and the like, tend to increase the liability to infection. In more subtle ways, our normal resistance is affected by the physiological state of the body. Any infection is likely to be more severe, for example, during pregnancy. As might be expected, natural resistance is lowered by malnutrition, particularly when the diet lacks some needed vitamin, by fatigue associated with overexertion and worry, by exposure to cold and wet, by alcoholism, and by preexisting weakening disease, such as cancer, diabetes, leukemia, measles, influenza, or tuberculosis. Age is also an important factor, the greatest susceptibility being among the very young (1 to 10 years) and among the very old.

The normal bactericidal power of the blood of different individuals doubtless varies, and probably it varies in the same individual from time to time. Some persons are able to mobilize their natural body defenses (inflammatory reaction, phagocytes) more promptly than others. There is also a difference in the rapidity and amount of antibody formation.

Acquired immunity. Importance as compared with natural immunity. The natural resistance to infection which we have been discussing is doubtless at its highest level in an individual without physical defects or physiological disturbances, that is to say, in an individual in a state of perfect health. But it must be remembered that this natural inherited immunity is not sufficient in itself to protect against most disease germs. The organisms of typhoid fever, influenza, syphilis, and other common diseases attack the physically strong and robust individual as readily as they do the weakling. Physical culture cannot of itself make the body immune to germs.

As we have explained in the previous chapter, successful resistance against germ diseases depends upon the development of a *specific immunity*, associated with the appearance in the blood of so-called antibodies which act upon the germ or its toxin. This specific resistance is *acquired* as a result of a natural infection, or by one of the artificial methods of immunization described below, and is probably *never inherited.*

Immunity acquired by recovery from germ diseases. Everyone is familiar with the fact that a lasting immunity follows recovery from certain diseases, for example, smallpox, measles, mumps, whooping cough, poliomyelitis, plague, typhoid fever, and yellow fever. In most cases this immunity appears to persist for the lifetime of the individual. Second attacks of some of these diseases may occur, but this is very rare.

On the other hand, there is only a very temporary resistance following pneumonia, influenza, gonorrhea, staphylococcus and streptococcus infections, and a few other diseases.

In *syphilis* a person is apparently resistant to a second attack so long as the original infection is still present. If an individual is reinfected, this is generally regarded as proof that the first infection had been cured.

In *tuberculosis* the situation is similar. Here again the infected individual develops a considerable degree of resistance, but if the infection is fully eliminated he becomes, in time, as susceptible as ever.

Immunity acquired by carriers. Many adults have a specific immunity to diphtheria, typhoid fever, and other diseases, though they have never suffered, to their knowledge, an attack of the disease. It is highly probable, however, that this immunity is in every case acquired (not inborn), as a consequence of some unconscious contact with the germ. They have perhaps had a very slight unrecognized illness, or they have been a *carrier* of the organism. In the latter case, the germ, or toxin, must have gotten into the tissues in sufficient amount to stimulate the development of specific antibodies, though it may never have produced noticeable symptoms. The development of immunity by carriers is generally regarded as the explanation of the fact that, as a rule, there are more people immune to diphtheria, for example, among the adult population in our crowded cities than in country districts. Country people are less often exposed to the germs, for they have fewer contacts with other individuals; there are fewer carriers, and therefore, fewer persons become immune.

Artificially acquired immunity. Active and passive immunization. There are two ways in which immunity may be acquired by artificial means: (1) by the introduction of a *vaccine*, or (2) by the injection of an *immune serum.* The former brings about *active immunization*, and the latter *passive immunization.*

(1) *Vaccines (active immunization).* The dead or weakened germ, or toxin, may be introduced into the body in the form of a *vaccine* for the purpose of stimulating an individual to develop specific antibodies. The process of producing immunity in this way is called *active immunization.* The individual is said to have an *active immunity*, because this immunity is the result of

his own activity in manufacturing antibodies. (The resistance which follows a natural infection is an active immunity.) In general, the immunity induced by active immunization is of a high degree and tends to be lasting.

(2) *Serums (passive immunization).* A temporary immunity may be acquired by injecting into the body an *immune serum*, *i.e.*, the blood serum of an immune animal or man. Production of immunity in this way is called *passive immunization.* The individual is said to have a *passive immunity,* because he took no active part in the development of his resistant state, but merely has received an injection of a serum *already containing the antibodies desired.* This immune serum was itself produced by the *active* immunization of another animal or man. Thus, diphtheria antitoxic serum is prepared by making a series of injections of diphtheria *toxin* into horses (Fig. 62). The animals are thus actively immunized, and their blood serum contains *antitoxin* (Fig. 63). When we take this immune serum and inject it into a human being, we are conferring upon this person a passive immunity. Passive immunity is temporary because the injected serum, being foreign to the body, is soon eliminated.

REVIEW QUESTIONS—CHAPTER XXIV

1. Define *immunity, natural immunity, acquired immunity.*
2. Discuss and give an example of congenital natural immunity.
3. What is meant by species immunity? Give examples of differences in susceptibility among animal species.
4. Name seven animal diseases transmissible to man. Name eight human diseases that do not occur in animals. What kinds of animals, in general, are most susceptible to human diseases?
5. Give examples of racial immunity among (*a*) animals, (*b*) human races.
6. Discuss immunity shown by a group of people considered as a whole.
7. Discuss the differences in susceptibility shown by different individuals. What explanation can be offered for some of these differences?
8. What is the importance of acquired immunity as compared with natural immunity? Is a specific immunity to a germ disease naturally inherited?
9. State the two ways in which immunity may be acquired.
10. Discuss and give examples of immunity acquired by recovery from germ diseases.
11. Discuss and give examples of immunity acquired by carriers.
12. In what two ways may immunity be acquired artificially? Define and give examples of (*a*) *active immunization,* (*b*) *passive immunization*

CHAPTER XXV

PRACTICAL USE OF VACCINES AND IMMUNE SERUMS

USE OF VACCINES. ACTIVE IMMUNIZATION

THE TERM *vaccine* was originally applied by Jenner (1798) to the preparation of *cowpox virus* which he used to immunize—*i.e.*, *vaccinate*—human beings against smallpox. The word was quite appropriate because *vacca*, in Latin, means *cow*. When Pasteur rediscovered the principle of protective inoculation and successfully applied it to the prevention of anthrax in cattle, and rabies in human beings, the materials he used for immunization were of quite different nature than the smallpox vaccine. Nevertheless, these preparations were also called *vaccines* and the process of immunization was spoken of as *vaccination.* So, while the term *vaccine*, in a strict sense, refers only to the cowpox virus as prepared for use in immunizing against smallpox, it is now commonly applied in a general sense to any material used for active immunization. *Vaccination* and *active immunization* now have essentially the same meaning.

Whatever its nature or origin, *a vaccine always contains a bacterium or a virus (killed or attenuated), or a toxin (in some way rendered harmless), and it is always introduced into the body for the purpose of stimulating the formation of specific antibodies and the development of a state of active immunity.*

Bacterial vaccines. A bacterial vaccine is merely a suspension of a culture of bacteria in physiological salt solution. In all the commonly employed vaccines, the bacteria are *killed*, usually by heating to about 60° C., and a small amount of a chemical disinfectant, such as tricresol (0.25 per cent), is added to the suspension as a preservative.

The usual method of preparing a bacterial vaccine is as follows: The organisms are grown in pure culture on slants of agar or other medium. The young growth is washed off in sterile physiological salt solution, with care to avoid contamination, and the resulting suspension is transferred to a sterile tube. The organisms are then killed by heating the tube in a waterbath at 60° C. to 65° C. for an hour. Sometimes a small amount of *formalin* is used instead of heat. A loopful of the suspension is now inoculated upon a suitable medium to see if all the organisms have been killed. In order to *standardize* the vaccine, it is necessary to determine the number of bacteria in a cubic centimeter of the suspension. This is usually done by mixing equal volumes of freshly drawn blood and the bacterial suspension in a pipette, spreading the mixture on a slide, drying, and staining the smear. The proportion of bacteria to red blood cells in the smear is determined by actual counting. Knowing that the number of red blood cells in a cubic *centimeter* of blood is five billion, and having determined the proportion of bacteria to blood cells, it is easy to calculate the number of bacteria in the suspension. Thus, if there are 5 red blood cells to every bacterium, the suspension contains approximately one billion bacteria per cubic centimeter. The suspension is diluted with salt solution to the desired concentration, about 0.25 per cent tricresol is added, the now finished vaccine is placed in sterile vials, and is ready for use.

Sometimes vaccines are made not from a single bacterium, but from mixtures of several different germs, such as staphylococci, pneumococci, and other organisms commonly associated with respiratory inflammations. Such a vaccine is called a *mixed vaccine.*

When bacterial vaccines are made from laboratory stock cultures, they are called *stock vaccines.* Better results are usually secured, especially in treatment, by the use of so-called *autogenous vaccines.* These are vaccines made from cultures of bacteria freshly isolated from the patient to be treated. Thus, an autogenous vaccine for furunculosis would be prepared by first cultivating the staphylococci from the boil, then making a vaccine from this same culture.

Other types of vaccines. The vaccines used for the prevention of smallpox, and of rabies, are examples of vaccines containing active *viruses,* whose virulence for human beings has been reduced.

Still another type of material used for active immunization is that employed for prevention of diphtheria and scarlet fever. In these diseases resistance depends upon a specific antitoxin, hence active immunity is induced by injections of the respective exotoxins, either mixed with antitoxin (so-called *toxin-antitoxin*) or in the form of *toxoid, i.e.*, toxin made nonpoisonous by treatment with formalin.

Therapeutic use of vaccines. Vaccines have been tried for the treatment of many different diseases. Opinions differ widely as to their value. Of course, the vaccine used in many instances does not contain exactly the same germ as that causing the illness and, therefore, the antibodies which are formed as the result of the treatment are not the ones that are really needed. Although good results have been claimed for vaccine treatment of acne, whooping cough, localized chronic infections like cystitis, and numerous other disease conditions, the true value of vaccine therapy is doubtful. Probably the most consistent success has been observed in the use of autogenous staphylococcus vaccines for the treatment of furunculosis.

Sometimes the injection of a vaccine has an indirectly favorable effect. It may cause a general reaction in the body which stirs all the defensive mechanisms into greater activity. Nonspecific vaccines, *i.e.*, vaccines containing bacteria other than those causing the infection, are sometimes used for this purpose and with good effect. A similar result can be obtained by the injection of various harmless substances containing foreign protein material.

Prophylactic use of vaccines. Principal diseases successfully prevented by vaccination. Vaccines have their greatest usefulness for the *prevention* of disease. They are of very great value as prophylactic agents for the immunization of well persons or animals *before* exposure to certain diseases, or before symptoms begin. The best known examples of human diseases successfully prevented by vaccination are *typhoid* and *paratyphoid fevers, smallpox, rabies, diphtheria,* and *scarlet fever.*

A definitely increased protection toward *cholera, plague, typhus, yellow fever,* and *whooping cough,* and toward the spirochetal diseases *yaws* and *Weil's disease* may follow injection of vaccines made from the causative organisms.

Typhoid and paratyphoid vaccine. This is a bacterial vaccine made up of killed typhoid and paratyphoid bacilli. Three injections of the vaccine are usually given subcutaneously about a week apart. This confers an apparently high degree of immunity to typhoid and paratyphoid infections which probably lasts for at least three years.

Smallpox vaccine contains the living virus of cowpox. To make the vaccine, cowpox virus is inoculated onto the abdomen of healthy calves, and the lymph which forms in the lesions is collected, mixed with glycerine, and stored for about a month, during which time most of the bacteria in it die. The ripened vaccine is then carefully examined for the presence of dangerous bacteria, such as tetanus bacilli. When the safety of the vaccine is assured and its potency has been established by animal tests, it is put upon the market in small capillary tubes for individual doses. The vaccine is inoculated into human beings by rubbing it into a small scratch in the skin, or by the so-called multiple-pressure technique.

Recently, vaccines made from preparations of the cowpox virus grown on the chorio-allantoic membrane of the chick embryo, or in tissue cultures, have been tried.

Rabies vaccine. As originally prepared by Pasteur, and as still frequently employed, this vaccine contains the active, but attenuated, virus of rabies. The vaccine consists of an emulsion in salt solution of small pieces of the spinal cord (containing the weakened virus) from rabbits dead of rabies. Recently, vaccines containing the virus *inactivated by phenol* have been widely used.

A series of daily injections of the vaccine are given, during a period of about three weeks, to persons who have been bitten by rabid animals. The unusually long incubation period in human rabies gives an opportunity to carry out this immunization before symptoms appear. If the vaccination is begun promptly after the bite, it is practically always successful in preventing rabies.

Toxin-antitoxin and toxoid for active immunization against diphtheria. The purpose of active immunization against diphtheria is to cause the development of diphtheria *antitoxin* by the individual, hence the vaccine must contain diphtheria toxin in some form. By one method of immunization injections of a mixture of diphtheria toxin and diphtheria antitoxin are made. Several different methods of preparing this *toxin-antitoxin* mixture are used, but in any case the mixture always contains a slight excess of free, unneutralized toxin. This free toxin acts as the stimulus for the formation of antitoxin *by the individual's own tissues, i.e.*, it causes the development of an active immunity against diphtheria. A better method of immunization, especially for young children, employs a vaccine consisting of diphtheria toxin which has been rendered nonpoisonous by treatment with formalin. This is called *diphtheria toxoid.* It is even more effective than toxin-antitoxin and has several important advantages in its favor. These methods of active immunization have proven highly successful in the prevention of diphtheria.

Immunization against other toxins. A toxoid made by treating tetanus toxin with formalin is now used for active immunization against *tetanus.* Similar toxoids prepared from the toxins of staphylococci, dysentery bacilli, and other bacteria have only a limited value as vaccines. Active immunization against *scarlet fever* is successfully accomplished by injections of the small amounts of the scarlet fever streptococcus toxin itself. These toxoids and other materials used for vaccination (active immunization) against toxins are properly called vaccines, though the student will find that in popular parlance this term is seldom applied to them.

Prophylactic immunization in other human diseases. Attempts have been made to build up a resistance against many other human diseases by vaccination, but without the conspicuous success that has followed active immunization against the diseases already mentioned. A vaccine for the prevention of *whooping cough* has recently given very promising results. Protection is afforded in many instances by preventive inoculation against yellow fever, Rocky Mountain spotted fever, and typhus fever. Vaccination is being seriously tried for dysentery, pneumonia, and influenza, and a few other common diseases. The so-called *BCG vaccine for tuberculosis* has had extensive trial, principally in Europe. This vaccine contains living bovine tubercle bacilli whose virulence has been lost by many years of cultivation on a certain culture medium. It is fed to infants in tuberculous families with the purpose of actively immunizing them against tuberculosis. Its efficiency is as yet uncertain.

Anthrax vaccine and other vaccines used for prevention of disease in animals. The anthrax vaccine is made from attenuated anthrax bacilli according to the methods worked out by Pasteur. The organisms are grown for some time (2 to 6 weeks) at an abnormally high temperature, 42.5° C. As a result of this treatment the bacilli no longer form spores and their virulence is greatly reduced. The vaccine is a suspension of these attenuated anthrax organisms. It is highly successful in preventing anthrax in cattle and sheep.

Several other vaccines are widely used by veterinarians for the immunization of animals—for example, vaccines for hog cholera, hemorrhagic septicemia and blackleg in cattle, and rabies in dogs.

Precautions in the use of vaccines. Vaccination substitutes for an attack of the disease itself a harmless contact with a germ or a toxin, as a result of which the body is stimulated to develop a specific resistance to that germ or that toxin. The procedure of vaccination necessarily involves a slight risk, but this is altogether insignificant in comparison with the great benefits it gives to the vaccinated individual. Of course, the actual inoculation of a vaccine, or for that matter of any material, must be performed with strict aseptic precautions.

The vaccines most widely used, such as those for the prevention of smallpox, diphtheria, and typhoid fever, are intended to be given to healthy persons able to withstand whatever reactions may follow the injections without harm. As a matter of fact, in the great majority of instances there is no general reaction of any consequence. On the second or third injection of typhoid vaccine, there is sometimes a reaction, but this is not serious, and is, in fact, a sign that immunity is being developed. Repeated injections of a vaccine, as in the preventive vaccination against rabies, sometimes causes a mild and transient inflammation at the site of previous injections. This inflammation is explained by the fact that the tissues have become sensitive to the injected material (see the following chapter).

On the ninth or tenth day following inoculation of smallpox vaccine, there is sometimes a mild general reaction, with fever and malaise, but otherwise the general health is not in the least disturbed. Of course, the vaccination sore is really a wound, and like any skin wound is liable to be infected from without unless properly cared for, but the smallpox vaccine itself is absolutely free of any harmful germs. The special precautions necessary for the proper care of the vaccination wound are discussed in Chapter XXXIX.

Universal vaccination. Active immunization against smallpox and diphtheria should be recommended for *every child.* These immunizations are best administered at about the age of eleven or twelve months. The success of the immunization against diphtheria should be checked by a Schick test about six months later. Consideration should be given, also, to the advisability of vaccinating infants (at about the sixth month of age) against whooping cough, and older children against tetanus, and possibly scarlet fever and typhoid fever.

USE OF IMMUNE SERUM. PASSIVE IMMUNIZATION

Nature of immune serums. Differences between serums and vaccines. An immune serum is the blood-serum of an individual (animal or man) highly immune to an infectious disease. It contains, already formed, *antibodies* for the bacterium, virus, or toxin of that disease. In concentrated, purified state it is used for the passive immunization of another individual.

The student should be careful to avoid the indiscriminate use of the words *vaccine* and *serum.* A serum is literally *blood-serum,* but a vaccine is a totally different substance and is used with a different purpose. The essential constituent of a vaccine is the germ or toxin in it which acts as an *antigen* and stimulates the formation of specific antibodies. The essential constituents of an immune serum are the *antibodies* it contains. Vaccines are principally used for the *prevention* of disease through the production of a slowly developing, but lasting, active immunity in healthy persons before they are exposed to the infection Serums, on the other hand, are of greatest value for the *treatment* of acute disease, although they are also useful in some instances for the prevention of illness when an immediate protection is needed. The immunity produced by an injection of an immune serum is of brief duration—a few weeks at most—because the foreign serum, together with the antibodies it contains, is soon eliminated from the body.

Preparation of commercial immune serums. Most of the immune serums commonly used are prepared commercially by the immunization of horses (Fig. 62), except those for treatment of pneumonia, which are now made also from rabbits.

Antitoxin serums (for example, diphtheria and tetanus antitoxin) are manufactured by inoculating the horses with increasing doses of the respective *exotoxins* only (not the bacteria), and the only type of antibody in the serum is the specific antitoxin. When, after several injections during weeks or months, the horse has developed a high content of antibodies in his blood, the animal is bled from the jugular vein, with strict precautions to avoid contaminations. After the blood has clotted, the clear serum is collected (Fig. 63).

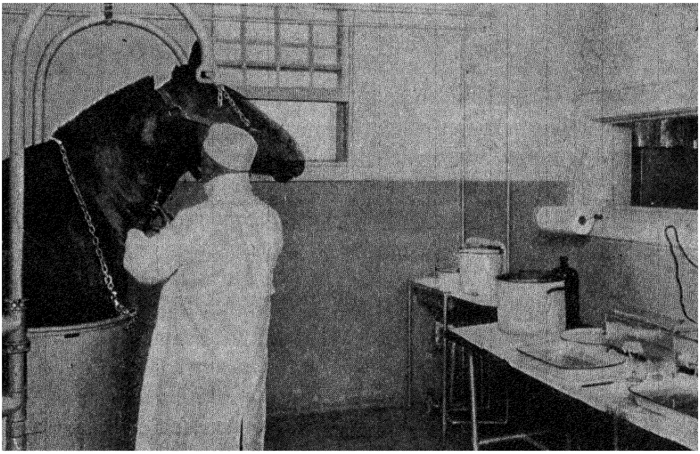

Fig. 62. Inoculation of a horse for the production of immune serum. When horses are being immunized to produce certain kinds of immune serum, for example, antipneumococcus and antimeningococcus serum, a suspension of the organisms is inoculated directly into the blood stream by way of the jugular vein, as shown here. Animals used for the production of antitoxin are inoculated with the toxin (or toxoid) subcutaneously and intramuscularly. (Courtesy of Parke, Davis & Co., Detroit.)

This serum is now purified and concentrated by separating from it the globulin portion which contains most of the antibodies, and discarding the remainder. This process eliminates a large proportion of the native horse protein and concentrates the antibodies so that a much smaller amount of the concentrated serum will be needed to get the same results. The only harmful reactions which may follow the injection of serum are due to sensitiveness to this foreign (horse) protein, and therefore it is very important to reduce this element of the serum as much as possible. The serum is made sterile by filtration, and a small amount of a chemical preservative is usually added. It is then standardized so that the physician may know the dose he may need to give.

Antibacterial serums (for example, antipneumococcus and antimeningococcus serums) are prepared in a similar way by immunizing the horses (or rabbits) with pure cultures of the bacteria, and the serums contain no antitoxin, but specific agglutinins, bacteriolysins, and other antibodies which act directly upon the germs.

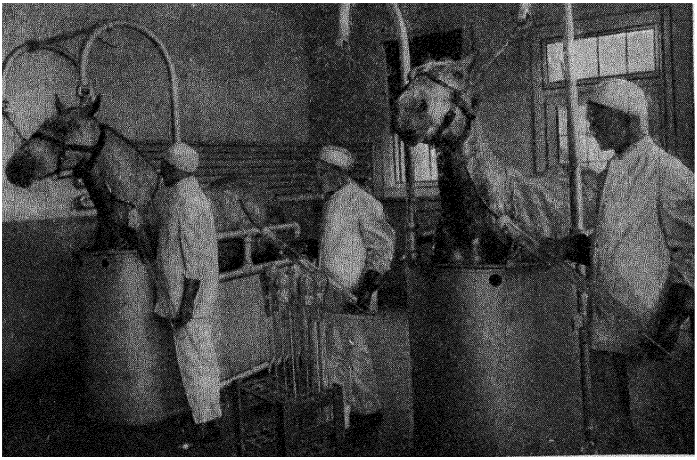

Fig. 63. Bleeding of horses after they have been immunized. A sterile needle is inserted into the jugular vein, and the blood is allowed to run into sterile glass cylinders. Note that careful aseptic technique is employed. The blood is allowed to clot in these tall cylinders, then the clear serum which has separated from the clot is collected. This serum, after concentration, purification, and standardization is ready for use in treatment of human beings. (Courtesy of Parke, Davis & Co., Detroit.)

In the United States, antitoxin and other immune serums, and similar biological products, such as smallpox vaccine, diphtheria toxin for Schick tests, scarlet fever toxin for Dick tests, tuberculin, etc., are manufactured only in establishments licensed by the Government, and under the supervision of the United States Public Health Service (National Institute of Health, Washington, D. C.). Frequent inspections of the plants and their methods are made to insure the reliability and safety of their products.

Principal commercial immune serums successfully used in prevention and in treatment of common diseases. The principal serums in common use are the *antitoxins* for *tetanus, diphtheria,* and *scarlet fever,* and *antibacterial serums for pneumonia and epidemic meningitis.* Serums are used also with varying success for the treatment of streptococcus infections, dysentery, anthrax, and some other diseases. An antitoxin for the toxin of the *Clostridium botulinum* is obtainable, but it is rarely possible to use this serum successfully in the treatment of botulism in human beings. An *antigangrene serum* for treatment of infections of accidental wounds (gas gangrene) has a limited use.

Tetanus antitoxin is an extremely valuable agent for the *prevention* of tetanus. About 1,500 units of the antitoxin are given subcutaneously to persons who have suffered a deep or lacerated wound which might lead to tetanus. This prophylactic dose is practically always successful in preventing the development of tetanus even in the case of severely wounded soldiers. Sometimes a *second injection* is necessary when wounds are slow to heal, because the protection afforded by one injection lasts only about two weeks. (*Active* immunization with tetanus *toxoid* will probably be employed as the preventive measure in the near future in place of this passive immunization with antitoxin.)

If symptoms of tetanus have already appeared, the antitoxin is of slight value, for it cannot undo the harm the toxin has already caused. Nevertheless, when the serum is given in large amounts before the disease gets too far advanced, it will sometimes save the life of the patient.

Diphtheria antitoxin. This was the first immune serum to be used on a large scale (beginning in 1894) for the treatment of disease and it still remains the most effective serum yet developed. It may be used successfully either for the prevention or the treatment of diphtheria.

A *prophylactic dose* of about 1,000 to 1,500 antitoxin units is given subcutaneously or intramuscularly to persons who have been exposed to the germs by contact with a case or a carrier, and are Schick-positive, i.e., susceptible, and are therefore in imminent danger of coming down with the disease. This injection gives these persons the immediate protection which they need.

Diphtheria antitoxin is universally used for the *treatment* of diphtheria and it is a certain cure if given early in the disease and in sufficient amount. The *therapeutic dose* varies according to the age of the patient and the severity of the illness from 3,000 to 30,000 or more units of antitoxin.

Scarlet fever antitoxin. This contains antitoxin for the toxin of the scarlet fever streptococcus, *Streptococcus scarlatinae.* Like diphtheria antitoxin it may be used as a prophylactic agent for susceptible persons who are in need of immediate protection. And also it may be successfully employed for the treatment of clinical scarlet fever. Though it has little effect upon the invading streptococci themselves, it does relieve the patient of his toxemia and strengthens his resistance, so that the danger of serious complications is greatly reduced.

Antipneumococcus serums. Most valuable of all the antimicrobial serums are those used in treatment of acute lobar (*Pneumococcus*) pneumonia. These are all type-specific antiserums, i.e., each is effective against only one particular type of pneumococcus, and the serum is administered to the patient after the type causing the illness has been determined. For some reasons not entirely clear, serums prepared against Type III pneumococci do not have sufficient protective power to warrant their use, but those made by immunizing horses (or *rabbits*) against Type I and Type II organisms are highly effective when given in the first three days of the disease. Recently it has been established that serums made by immunizing *rabbits* against many of the numerous higher-numbered types of pneumococci (IV–XXXII) also have definite therapeutic value.

Antimeningococcus serum. This serum is made by immunizing horses with a mixture of several different strains of meningococci isolated from different sources, and representing all the known types of this organism. This is done so as to have in the serum antibodies for all of the strains likely to cause a case of meningitis. The general use of this serum has brought about a reduction of more than 50 per cent in the death rate from epidemic meningitis.

Convalescent serum. In the case of a few human diseases it is now a common practice to use for prevention or treatment the *blood serum of another person who is convalescing* (or has recently recovered) from that disease. Such serum is spoken of as *convalescent serum. Measles* and *scarlet fever* are the diseases in which convalescent serum has been most often employed. When serum from a child just recovering from measles is given to a child just coming down with measles, the disease is made much milder; or if the serum is given early enough, it may prevent the disease entirely. Convalescent serum may be used either for the prophylaxis or therapy of scarlet fever, instead of commercial serum.

Precautions in the use of serum. Just as in the case of vaccines, the risks involved in the use of serums are insignificant in view of their proven capacity to prevent illness and to save lives. The dangers associated with injection of commercial serums arise entirely from the fact that the serum itself—without regard to the antitoxin or other antibodies it may contain—is protein material (usually horse serum) which is foreign to the human body, and some individuals may have a *hypersensitiveness to this foreign protein.* The injection of serum in a single large dose in an individual naturally very sensitive to horse protein may produce severe symptoms or even sudden death. But such cases of natural hypersensitiveness to horse serum are very rare. A person may acquire this sensitiveness, however, as a result of a previous injection of horse serum, so that if at a later period (two weeks or more after the first injection) he must receive a second injection, there is danger of a reaction. In the majority of individuals, including those not abnormally sensitive, there develops after the injection of a large amount of serum a condition known as *serum sickness.* This is not serious and does no permanent harm. All of these reactions after the use of serum are manifestations of a condition of hypersensitiveness to the protein constituents of serum. The phenomena of hypersensitivity (anaphylaxis) are discussed in the following chapter.

The possibility that the patient is hypersensitive must be taken into account whenever serum is to be administered, but this does not mean that serum treatment can not be given when it is needed. There are various ways by which the existence of a sensitive state can be detected before any damage is done, and also there are ways of administering serum safely even to sensitive individuals. Hence, it is only necessary for physicians to guard against the possible danger of hypersensitiveness, for otherwise serums may be used with complete safety.

REVIEW QUESTIONS—CHAPTER XXV

1. What was the original meaning of the word *vaccine* and how did it come to have its present use?
2. Write a definition of a vaccine which will show its content and purpose. Define *vaccination.*
3. Define *bacterial vaccine, mixed vaccine, stock vaccine, autogenous vaccine.* Explain how bacterial vaccines are usually prepared.
4. What other types of vaccines are commonly used?
5. Discuss the therapeutic use of vaccines.
6. Name four diseases successfully prevented by prophylactic vaccination (active immunization).
7. Describe briefly the nature and use of typhoid and paratyphoid vaccine, smallpox vaccines, rabies vaccines, and toxin-antitoxin and toxoid for diphtheria.
8. Name two other important diseases which may be prevented by active immunization against the toxins concerned.
9. Mention other germ diseases, prevention of which by vaccination is attempted.
10. Describe the anthrax vaccine and name other vaccines used for prevention of disease in animals.
11. Discuss precautions and dangers in the use of vaccines.
12. What are the two diseases against which every child should be vaccinated?
13. Explain clearly the differences in the nature, use, and purpose of (1) vaccines, and (2) serums.
14. Outline briefly how commercial antitoxins and antibacterial serums are prepared. What is the purpose of purifying, concentrating, and standardizing the serum? What agency acts to insure the reliability and safety of commercial immune serums?
15. Name five immune serums most widely used in prevention or in treatment of common diseases. Name five other immune serums of more limited use or value.
16. Describe briefly the nature, use, and value of tetanus antitoxin, diphtheria antitoxin, scarlet fever antitoxin, antipneumococcus serum, and antimeningococcus serum.
17. What is meant by *convalescent serum?* Name two diseases in which it may be successfully used.
18. Explain the dangers associated with the injection of foreign serum into the body.

CHAPTER XXVI

ANAPHYLAXIS OR HYPERSENSITIVENESS

CLOSELY ASSOCIATED with immunity is the interesting and important condition called *anaphylaxis or hypersensitiveness.* The term *anaphylaxis* means, literally, the "contrary of protection." It was originally coined to signify the opposite condition from *prophylaxis,* which means "protected" (by preventive methods). It is not true, however, that the anaphylactic individual is without protection, only that in addition to any powers of resistance toward bacteria or other harmful agents he may have, he is in a state of abnormal susceptibility, or sensitivity, i.e., he is *hypersensitive* toward a germ or its products or toward a foreign substance of some other kind.

Anaphylaxis is a subject of much theoretical interest because of its relationship to immunity. It is also a matter of great practical importance because there are several common forms of illness in human beings which are due to a condition of hypersensitiveness. Some individuals, for example, are naturally hypersensitive to the pollen of the ragweed, or other plants, and suffer annually an attack of hay fever, or asthma, when these pollens are abundant in the air. Other individuals are sensitive to certain foods, or drugs, or other substances, and some skin disorders, gastro-intestinal disturbances, and other pathological conditions, are anaphylactic in nature. In the course of various infections, hypersensitiveness of the tissues probably accounts for many of the characteristic lesions and symptoms of the disease. Of the greatest importance from the practical point of view are the anaphylactic reactions which may follow the deliberate injection into human beings of foreign substances, such as vaccines, and particularly serums.

In order to understand hypersensitiveness in man, it will be necessary first to describe the principal features of anaphylaxis as observed in laboratory animals.

ANAPHYLAXIS IN ANIMALS

Although hypersensitiveness in animals had been noted by several workers some time before, the subject gained particular attention as a result of the observations of Theobald Smith (1904). In the course of some studies of diphtheria antitoxin, Smith accidentally discovered that guinea pigs which had received an injection of the antitoxic (horse) serum *became, after an interval of about twelve days or more, highly susceptible to a second injection of plain horse serum.* Within a few minutes after the second injection of the serum the animals became acutely ill, with characteristic symptoms, and most of them died in a very short time. They suffered what is now called an acute *anaphylactic shock.*

Careful studies of this remarkable occurrence, and other forms of anaphylactic reactions in animals have since been made by a great many investigators, and the most important features of anaphylaxis are now well known, although authorities are not yet agreed as to the correct theoretical explanation of the phenomenon.

Principal features of anaphylactic reactions in animals. The most important facts concerning anaphylaxis as it has been observed in laboratory animals are summarized in the following paragraphs:

(1) A state of hypersensitiveness can be induced in animals by the injection of *any foreign protein material, i.e., any antigen*—for example, perfectly harmless substances like egg-white, milk, or blood serum, as well as bacteria, etc., etc. (The injection of an antigenic substance leads, of course, to the development of specific *antibodies*).

(2) An anaphylactic reaction may follow the second (or later) injection of the particular protein, *whenever the interval between injections is ten to twelve days or more.* The animal does not become hypersensitive until an interval of about ten days has passed since the first or sensitizing dose was given.

(3) Hypersensitiveness is highly *specific,* just as immunity is specific. An animal sensitized to one protein reacts only to subsequent inoculation of that identical protein, and not to other substances.

(4) A single injection of a very minute amount of a foreign

protein may suffice to produce a highly sensitive state in some animals, notably the guinea pig. Rabbits and some other animals are not so easily sensitized. Among individual animals of the same kind there are marked differences in the ease with which they can be sensitized. Once an animal has become hypersensitive to a certain substance, it will remain so for months and years, and probably for the rest of its life. However, the degree of sensitiveness becomes gradually less after the first month or two.

(5) A relatively large amount of the antigen must be inoculated into the hypersensitive animal in order to produce a typical anaphylactic shock—perhaps 100 times as much as was required to sensitize. *Smaller amounts may cause only a slight reaction or none at all.*

The rate at which the foreign substance enters the circulating blood is also of very great importance in determining the severity of the reaction. *The acute and severe anaphylactic reactions are usually brought about by a sudden introduction into the circulation of a considerable amount of the foreign material.*

(6) It follows, from the statements just made, that anaphylactic reactions can usually be prevented if the specific antigen is introduced into the hypersusceptible animal in such a way that it reaches the general circulation *slowly and in very small amounts.* By such a process a hypersensitive animal may be *desensitized.* Thus, if the sensitive animal is first given an injection of a very minute amount intradermally, or subcutaneously, so that the material will be absorbed only slowly, then at half hour intervals further injections are made, it is possible to introduce, without producing anaphylactic reactions, a total amount far in excess of the amount necessary to kill the animal if injected in a single dose.

(7) If an animal survives an anaphylactic attack, it becomes resistant or refractory to further injections of the same substance for a variable period of time thereafter, though it eventually becomes sensitive again. This temporary lack of sensitiveness following recovery from an anaphylactic reaction is spoken of as *anti-anaphylaxis.*

(8) *Passive hypersensitiveness* can be induced in a normal animal, just as passive immunity is conferred, by injecting into the normal animal some of the blood serum of the hypersensitive animal. As a rule, in guinea pigs, but not in mice or rabbits,

a period of a few hours must pass before the injected animal becomes sensitive. If, then, an injection of the specific substance is given, this passively sensitized animal will show a typical anaphylactic reaction.

(9) The *symptoms* of anaphylaxis are always the same in animals of the same species, irrespective of the nature of the substance to which the animal is sensitive. But the anaphylactic reaction differs in different species of animals. It differs according to the anatomical and physiological makeup of the animal, not according to the chemical nature of the sensitizing substance.

In the *guinea pig* the symptoms of acute anaphylactic shock usually begin within a minute or two after the material to which the animal is hypersensitive has been injected. The animal becomes very restless and agitated, and soon it is apparent that it is having a great deal of difficulty in breathing. It rubs its nose, coughs, and often rises on its hind legs or makes a series of jumps in a violent effort to get air. Within a few minutes the animal is no longer able to stand; it falls upon its side, gasping for breath. Sometimes a slow recovery takes place, but usually convulsions set in and breathing soon ceases. The heart continues to beat for several minutes after breathing has stopped. On autopsy the lungs are found distended and filled with air. This is due to the fact that the smooth muscle tissue about the bronchi and bronchioles has contracted, so that the passages for air are almost completely closed off. The devitalized air in the lungs could not be expelled and fresh air could not be drawn in, and as the inevitable result of this obstruction to respiration, the animal suffocates.

In *rabbits* respiratory symptoms are not prominent and death is not due to suffocation, but is explained by a spasmodic constriction of the walls of the pulmonary arteries, causing a rapid dilatation of the right side of the heart, and sudden heart failure.

In *dogs* the symptoms are more varied, but are referable principally to the disgestive tract. There is a marked fall of blood pressure, and a collection and stagnation of blood in the tissues, especially in the liver.

(10) A very prominent feature of the acute anaphylactic reaction is the spasmodic contraction of *smooth* (involuntary) *muscles.* It happens that, in guinea pigs, the bronchi and bronchioles are especially rich in this smooth muscle tissue. This

explains why anaphylactic shock in these animals is characterized principally by difficulties in respiration and eventual suffocation. In other animals the smooth muscle is less abundant about the bronchi. It is probable that the differences in the symptoms and lesions of acute anaphylaxis as observed in different species of animals may be due principally to differences in the amount and distribution of smooth muscle tissue in the various parts of the body.

The fact that in the hypersensitive animal the smooth muscles are sensitized, and will be thrown into spasmodic contraction when the specific antigen reaches them, can be demonstrated by experiments upon a piece of such smooth muscle tissue (the uterus, or a strip of the intestine, for example), taken from the body of the animal. If the uterus of a hypersensitive guinea pig is removed and immediately suspended in a bath of warm physiological salt solution, it will contract vigorously when a little of the substance to which the animal was sensitized is added to the bath.

(11) A very significant contribution to our understanding of anaphylaxis was made by Dale, when he showed that a substance called *histamine* will cause the symptoms of acute anaphylaxis when injected by itself into normal animals. Histamine is derived from the amino acid histidine. It may be formed during the decomposition of protein material. It is probable that many of the most characteristic features of acute anaphylactic shock, are the result of the liberation of histamine or a *histamine-like substance,* in the body fluids or in certain cells during the anaphylactic reaction.

(12) Anaphylactic shock can be prevented, and the symptoms of anaphylaxis can be relieved by the use of drugs, such as atropine, epinephrin (adrenalin), and ephedrine, which help to maintain blood circulation and tend to inhibit spasmodic contraction of smooth muscles, especially those of the bronchi.

HYPERSENSITIVENESS IN HUMAN BEINGS

The manifestations of hypersensitiveness in man differ in a number of respects from the typical anaphylactic reactions experimentally produced in laboratory animals as above described. These differences led some of the earlier workers in this field to believe that true anaphylaxis does not occur in human beings. It is now generally agreed, however, that, although very severe or fatal anaphylactic shock in man is rare, the sensitiveness shown by many individuals toward pollens, foods, drugs, and other substances, the reactions following injection of serum, and other forms of human hypersensitiveness are all fundamentally of the same nature as anaphylaxis in animals.

Allergy. On account of the disagreement among scientists as to the real nature of human hypersensitiveness, much confusion in terminology has arisen. One term now widely employed with reference to hypersensitiveness in man is *allergy*. This term is derived from the Greek *allos*, other or different, and *ergon*, work or action, and means literally the *different or altered power to react*. Thus, the hypersensitive individual is said to be *allergic*, because he reacts in a different way than the normal individual toward foreign matter of some kind. He will show an *allergic reaction* when exposed to contact with the substance to which he is sensitive.

If the term were used in its literal sense, *allergy* would include *immunity* as well as hypersensitiveness, because the immune individual also reacts toward foreign substances in a different way than the normal individual. In clinical medicine, however, physicians use the word *allergy* to mean the same as *hypersensitiveness*, and with special reference to the hypersensitivity observed in human beings.

Common forms of human allergy. Human beings may show a natural hypersensitiveness to a great variety of substances. Just how this sensitiveness originates is not entirely understood. Undoubtedly an *inherited tendency to become sensitized* plays an important part. Probably the hypersensitiveness arises in susceptible individuals as a result of absorption of foreign substances through the mucous membranes of the respiratory or digestive tract, and allergic reactions occur when the same substances are absorbed at a later time in the same way.

One class of substances to which many individuals are hypersensitive includes the *pollens* of certain weeds, grasses and other plants. These pollens are of the type that are borne about by the winds and become abundant in the air at certain seasons. So-called *hay fever* is most commonly due to hypersensitiveness to ragweed pollen. When the pollen grains are inhaled, they cause a more or less acute coryza, somewhat like an ordinary cold, and may produce symptoms of *asthma.* This distressing condition is often successfully prevented by a process of partial desensitization before the season begins. This is accomplished by a series of subcutaneous injections of very small amounts of an extract of the pollen given at intervals of a few days. The condition of partial antianaphylaxis thus produced is not permanent, and the individual must submit to the injections every year.

Asthmatic symptoms are caused in some individuals by the inhalation of particles of feathers, hair, or dandruff of certain animals, or by breathing in various other substances which may be present in the air.

Everyone is familiar with the fact that some persons cannot eat certain *foods* without developing a more or less severe illness, which may be characterized by gastrointestinal disorders, a skin rash, or asthmatic symptoms.

Skin rashes or other disturbances sometimes follow the taking of a certain *drug,* such as iodine, quinine, or mercury. These substances are not proteins and are not antigenic in themselves. It is believed, however, that some peculiar protein product of the tissues, which forms when the drug is taken frequently, acts as an antigen to produce specific antibodies and so may bring about a state of hypersensitiveness.

Just as in the experimental anaphylaxis in animals, the symptoms of these allergic reactions in man are essentially the same no matter what the nature of the sensitizing material may be. The symptoms are relieved by the same agents (adrenalin. ephedrine, etc.), which serve to prevent anaphylactic reactions in animals. Sometimes the hypersensitiveness almost entirely disappears when abnormal conditions of the mucous membranes, caused, for example, by a deviated nasal septum or chronically inflamed tonsils, are corrected, and some forms of food allergy may vanish as a person grows older. Presumably this improvement is due to a decrease in the amount of the foreign protein absorbed.

Anaphylactic reactions following the injection of vaccines. When several injections of a vaccine are given over a considerable period of time, as, for example, in the preventive vaccination against rabies, the local inflammation about the site of the inoculations, following the later injections, is undoubtedly due to the fact that the tissues have become sensitized to the vaccine. These local reactions are not serious, however.

More marked local or general anaphylactic responses sometimes develop when an individual who has once been vaccinated is revaccinated with the same vaccine some months, or even some years, later. Thus, a person who has received the usual three injections of typhoid vaccine will often show an almost immediate reaction of more or less severity if he later takes the vaccine a second time. These reactions pass quickly, however, and there are no serious consequences.

An interesting example of a modified response following a second inoculation of a vaccine is the so-called accelerated reaction which often occurs when an individual is re-vaccinated against smallpox some years after the first "take." Instead of the slow development of the vaccination sore, characteristic of the primary vaccination, which usually does not reach its height for about ten days, the accelerated reaction runs a more rapid course and is completed within seven or eight days (Fig. 95).

Anaphylactic reactions following injection of serum. Of the greatest practical importance are the anaphylactic reactions which may develop as a result of the inoculation of a foreign serum, such as a commercial antitoxin, into human beings. Of course these reactions are not due to the antitoxin, or other antibodies the serum may contain, but are caused by a sensitiveness to the serum itself. They usually take the form of so-called *serum sickness,* but rarely there occurs a severe or fatal *anaphylactic shock.*

Serum sickness. Most commonly reactions appear only after an interval of several days and are mild in character. This delayed response to the injection of serum is called *serum sickness.* A peculiarity of this condition is that it may develop not only in persons who have been sensitized by a previous injection of serum, but in a large majority of perfectly normal individuals after receiving an injection of a considerable amount (100 c.c. or more) of a serum for the first time. The illness is usually quite mild. Symptoms first appear in most cases in about eight to twelve days. They include a skin eruption and usually some fever, and tenderness and stiffness of the joints. There are no permanent ill effects. This delayed response is thought to be due to the reaction between the portion of the injected serum still present in the body and the antibodies to the serum itself which are actively forming as a result of the injection.

Acute anaphylactic shock. Whereas the mild serum reactions above described are common, acute anaphylactic shock is an extremely rare occurrence. Nevertheless it may occur, though only one individual in many thousands may be so hypersensitive as to be liable to develop so violent a reaction. Practically all of the reported cases of sudden death following a serum injection have occurred in persons *naturally* highly hypersensitive to horse dandruff or other forms of horse protein, and the shock developed immediately after the first injection of the serum.

Desensitization of individuals before injection of serum. Physicians make it a practice to precede the injection of the full dose of any serum by an *intradermal* inoculation of a very minute amount (say 0.02 c.c.) of diluted serum as a test for sensitivity. The unsensitive individual will show a small wheal at the point of inoculation which will disappear rapidly, but in the sensitive persons there will be a marked inflammatory reaction. Instead of this skin test the *ophthalmic* test may be employed, in which a little of the dilute serum is instilled into one eye. Again an inflammatory reaction develops at once if the individual is hypersensitive. These preliminary tests are especially important in the case of patients who give a history of sensitiveness to horse protein in any form, patients with asthma from any cause, and individuals who have received previous injections of horse serum.

If the patient proves to be hypersensitive by these tests, it is not necessary to deny him the benefit of serum treatment. It is only necessary that the physician exercise great care to administer the serum *very slowly* so as to avoid an explosive anaphylactic reaction. This is accomplished by giving very small amounts of the diluted serum subcutaneously at half hour intervals until one or two cubic centimeters of serum have been injected without harm. It is then usually safe to inject the remainder of the dose.

Hypersensitiveness in infectious diseases. Skin reactions and their use in diagnosis. Persons may become hypersensitive to bacteria, just as to other protein substances, and in the course of infectious diseases a general or localized hypersensitiveness may develop toward some substance contained in or produced by the bacterial cell. This is especially likely to occur in infections which are chronic, like tuberculosis. There is no doubt that many of the characteristic symptoms and pathological changes in many diseases are in part anaphylactic in nature. The hypersensitiveness which develops as a result of infection is sometimes utilized as a basis for diagnosis. If an extract containing the antigenic protein fraction or the hapten of the infecting organism is introduced into the skin, there will be a specific local reaction in the infected individual, but not in the normal individual.

The most widely used of such allergic skin tests is the *tuberculin test* for tuberculosis. Tuberculin is a product prepared from tuberculosis bacilli, and individuals infected with these germs become hypersensitive to it. When a small amount of tuberculin is injected or rubbed into the outer skin of a person who is infected, or at some previous time *was* infected with tuberculosis bacilli, a marked local reaction occurs. A noninfected person does not give this reaction.

Relation between hypersensitiveness and immunity. Theories of anaphylaxis. Most authorities now believe that the very same antibodies responsible for the condition of immunity are also concerned in the phenomenon of anaphylaxis. Though the effect upon the body as a whole is quite different, the fundamental occurrence in anaphylaxis, as well as in immunity, is the combination of the foreign protein (antigen), either in the circulating blood or in the body cells or both, with its specific antibody. Thus, immunity and anaphylaxis are but different phases or aspects of the same thing.

The real difference in the effects produced by the union of antigen and antibody in the immune individual, on the one hand, and the hypersensitive individual, on the other hand, may be explained by the difference in the *speed* with which physicochemical changes induced by this combination occur. In the hypersensitive state, antigen and antibody combine with great rapidity and the products formed by this reaction accumulate in large amounts within a short space of time. These products probably include poisons of the nature of histamine or related substances. It appears that more poison forms *within a limited time* than can be assimilated without harm, and the symptoms of anaphylaxis appear.

REVIEW QUESTIONS—CHAPTER XXVI

1. Give a literal definition of *anaphylaxis.* In a more general sense, what characterizes the anaphylactic state? What were the circumstances in which acute anaphylactic shock in animals was first observed?
2. Summarize the principal features of experimental anaphylaxis in animals (twelve main points).
3. Explain clearly what is meant by *desensitize, anti-anaphylaxis, passive hypersensitiveness.*
4. Give a literal definition of *allergy.* Explain the common use of the term.
5. Discuss briefly common forms of human allergy.
6. Under what circumstances may anaphylactic reactions follow the injection of vaccines? Give examples.
7. Why are anaphylactic reactions following injections of serum into human beings of great practical importance? What two types of reactions may occur?
8. Explain the nature of serum sickness. Under what circumstances is a mild serum sickness likely to develop? What circumstances may make the reaction more severe? Are there any serious or permanent ill effects from serum sickness?
9. Under what circumstances may a fatal anaphylactic shock occur following a serum injection?
10. Describe methods of testing for hypersensitiveness to serum. When are such tests advisable? What is the general procedure for giving serum treatment safely to a hypersensitive individual?
11. What imporance have the phenomena of hypersensitiveness in connection with infectious diseases? How may this hypersensitiveness be used as a basis for diagnosis? Give an example.
12. Discuss briefly the relation between hypersensitiveness and immunity and a possible explanation of anaphylaxis.

PART FIVE

MICROBIOLOGY OF IMPORTANT INFECTIOUS DISEASES

CHAPTER XXVII

INFECTION OF THE SKIN. STAPHYLOCOCCUS INFECTIONS

INFECTION OF THE SKIN

THE SURFACE of the skin of human beings is never quite free of bacteria, whether it be clean or dirty. Of course, if it is not clean, a great number and variety of micro-organisms may be present, including, perhaps, yeasts and other fungi, or protozoa, as well as bacteria. The hands, especially, are liable to acquire a large and varied population of both harmless and disease-producing organisms by contact with the dust and unclean objects of all sorts, and the fingers are liable to be contaminated with germ-laden saliva or excreta.

These accidental contaminations of the skin do not concern us here, however. The important fact is that *even the perfectly clean skin constantly harbors bacteria.* There are a few kinds of organisms which seem to multiply freely upon the healthy skin. These bacteria are to be found not merely on the superficial layers of the skin, but also in the deeper parts, especially about the hair follicles and sebaceous glands, and in the ducts of the sweat glands. Ordinary washing does not remove all these organisms, and even prolonged scrubbing will not sterilize the skin.

The most common types of these bacteria found constantly on the clean skin are Gram-positive cocci, and the majority of these belong to a single group—the *staphylococci.* In certain regions of the body several other types of bacteria are usually present. About the external genitals and anal region, for example, an organism almost always to be found is a harmless acid-fast bacillus called the *Mycobacterium smegmatis.* Similarly, on the skin lining the external opening of the ear diphtheroids (bacilli resembling the diphtheria bacillus in shape, but not pathogenic), and harmless acid-fast bacilli which live in the ear wax, are often present, as well as the usual staphylococci. It is the latter, however, that are the important members of the normal flora of the skin, for these cocci are "opportunists," and the most common cause of the familiar pimples or boils that appear where the normal skin is cut or abraded. There are many other forms of infectious skin diseases, however, and some of the pathological conditions due to causes other than staphylococci will first be mentioned.

Skin infections caused by fungi. Several infections involving the skin and caused by yeasts, molds, or moldlike organisms are common. The most important are *blastomycosis*, *sporotrichosis*, *ringworm*, and *actinomycosis*. These infections are described in Chapter XL.

Skin infections of uncertain cause. In many cases lesions on the skin are known to contain bacteria or fungi, but the relation of these organisms to the origin of the disease is uncertain.

Acne is a chronic inflammatory disease of the sebaceous glands in which papules or pustules form upon the face. An organism called the *Corynebacterium acne* is usually found in the skin lesions, but sometimes staphylococci appear to be the only organisms present. Frequently both the staphylococci and the "acne bacillus" are found. The latter may be cultivated, with difficulty, under anaerobic conditions. Because it somewhat resembles the diphtheria bacillus in shape and staining, it is classed as a *diphtheroid.*

Granuloma inguinale is a form of skin infection occasionally seen in the United States. In this disease there is a superficial *ulceration* about the genitals and neighboring parts, often involving a large area of skin. The infection has a long, chronic course. The true causative organism is unknown. (See Chapter XXXIV).

Decubitus ulcers, or bed sores, often develop as a distressing complication in patients who are obliged to lie in bed for long periods. These ulcers naturally appear at those places where normal circulation of the blood is interfered with by the weight of the body. They form most commonly in the sacral regions, and are especially liable to appear if there is incontinence of the urine or feces. No one particular germ is responsible for these ulcers, but a mixture of organisms, including staphylococci and several other kinds. These microbes cause a slow destruction of the devitalized tissues but do not ordinarily invade the deeper parts of the body. By keeping the skin clean and dry (alcohol rubbing), by the use of air cushions, and by frequently changing the position of the patient, the formation of these ulcers may often be prevented.

Specific bacterial diseases with lesions in the skin. The skin is involved either primarily or secondarily in several important germ diseases. In one of the principal clinical forms of *leprosy,* hard nodular swellings appear in the skin, principally upon the face. The organisms of *tuberculosis* sometimes localize upon the skin. The commonest form of skin tuberculosis is called *lupus.* The earliest lesion in *syphilis* is a hard sore called the chancre, which appears at the spot on the body where some minute break in the mucous membrane or skin has permitted the germ to enter. *Yaws,* a tropical disease resembling syphilis in many ways, has characteristic lesions upon the skin. In the course of *typhoid fever,* the germs of this disease may localize in the so-called "rose-spots" just under the skin. Human beings are sometimes infected with the germs of *anthrax* through a break in the skin. A sore develops called a "malignant pustule." When the organisms of *glanders* or *tularemia* get through the skin, a characteristic inflammatory lesion appears at the place of entrance.

Streptococcus infections. A variety of *Streptococcus,* closely related to the streptococci found in scarlet fever, causes *erysipelas,* a peculiar skin disease characterized by an acute, spreading inflammation of the superficial lymphatic vessels of the skin, usually without pus-formation.

Rarely streptococci of other types produce a localized *abscess in the skin* much like the ordinary boil caused by a staphylococcus, but usually streptococci are so virulent that, once through the skin, they immediately invade the deeper tissues, causing an acute spreading infection of the subcutaneous tissues, called *cellulitis,* with, finally, an invasion of the lymphatics and the blood, or an acutely fatal *septicemia* without any localization in the skin. The numerous infections which may be caused by streptococci are considered fully in a later chapter.

Virus infections. Several kinds of filtrable viruses cause infections which characteristically involve the skin. Among the more prevalent of these are *smallpox* (discussed in Chapter XXXIX), *chicken-pox*, *herpes simplex*, *herpes zoster*, and *common warts.*

Chicken-pox appears to be due to a virus, though filtrability of the infectious agent has not been demonstrated. The virus is present in the bacteria-free fluid from the vesicles. The virus particles, or elementary bodies, may be seen, when propriately stained, in this fluid, and characteristic intranuclear inclusion bodies are found in the affected epithelial cells.

Herpes simplex (or *febrilis*), manifests itself as small vesicles about the mouth or nose of patients who are suffering from severe colds, pneumonia, or other acute disease. A filtrable virus is always present in the local vesicles and sometimes can be recovered from other tissues. The affected tissue cells contain intranuclear inclusion bodies, and in the vesicular fluid the virus is in the form of stainable elementary bodies. In tissue cultures the virus grows readily. Herpes virus may cause a brain infection (encephalitis) in experimentally inoculated rabbits or mice, and is capable also of infecting the human brain, but it is not the usual causative agent of human encephalitis.

Herpes zoster differs from herpes simplex clinically in the size and location of the vesicles, which are large and appear mostly on the trunk and along the course of sensory nerves. There are accompanying neuralgic pains. Elementary bodies may be found in the vesicular fluid and there are intranuclear inclusions in the tissue cells. The virus is distinct from that of herpes simplex; it has not been filtered.

Common warts are caused by a virus which can be recovered by filtering ground-up wart material. Inclusion bodies are present in the skin cells.

STAPHYLOCOCCUS INFECTIONS

The pathogenic staphylococci. *Species and varieties and their distribution.* Several varieties of staphylococci have been described. There are only two species, however, which are important in connection with disease; these are called *Staphylococcus albus* and *Staphylococcus aureus.* The species names refer to the color of the growth of these organisms on agar media. Cultures of the *albus* species are white. The *aureus* species forms an orange or golden yellow pigment.

The staphylococci are constant inhabitants of the mucous membranes as well as the skin of human beings and animals. Since they are rather hardy organisms, and especially resistant to drying, they may often be found in the dust of inhabited rooms. The *albus* type is usually abundant there, and upon the clean skin and mucous membranes. The *aureus* species, on the other hand, is the kind found most commonly associated with pathological conditions.

Morphology and staining. The individual staphylococci are tiny spheres slightly less than one micron in diameter. In smears they are characteristically clustered together in irregular-shaped masses. Sometimes in smears from pus or pure cultures they appear arranged in groups of two or four cells, or in short chains, and it is often difficult to be certain from microscopic examination alone whether or not the organism is a staphylococcus. The cocci are nonmotile, and have no spores. They are readily colored with the common stains, and are Gram-positive.

Physiological properties. All varieties of staphylococci grow luxuriantly upon even the simplest culture media and they multiply at room temperature as well as at body temperature. They develop best in the presence of abundant atmospheric oxygen, but will also grow in partially anaerobic conditions.

The staphylococci are unusually resistant organisms. They remain alive in laboratory cultures without transplantation much longer than most disease germs. When dried on paper or cloth, they may be found alive after many weeks. They are also less readily killed by heat or by chemical disinfectants than other bacteria.

Products of growth. Toxin production. The staphylococci as a group, and the pathogenic strains in particular, are active in bringing about chemical changes. Most varieties are able to *liquefy gelatin*, a power few disease germs possess. Also, most virulent staphylococci elaborate fibrin-dissolving, and plasma-coagulating enzymes, and a substance called *leukocidin* which destroys leukocytes. The latter probably increases the disease-producing power of the cocci.

Certain pathogenic strains of *Staphylococcus aureus* form true exotoxins. The sterile filtrate from cultures of these toxigenic strains has (1) a hemolytic action, causing the hemolysis of red blood cells, (2) capacity to cause death and destruction (necrosis) of the skin of rabbits and other animals when inoculated intradermally, and (3) power to kill rabbits and mice almost instantly when inoculated intravenously. The part played by these toxic principles in human staphylococcus infections is uncertain, but probably is not as great as might be expected from their marked effect on animals.

Besides the toxic substances just mentioned, a few strains of staphylococci, when growing in suitable circumstances, form another sort of toxin, sometimes spoken of as an *entertoxin.* A filtrate from a culture of a staphylococcus of this kind produces gastro-intestinal symptoms when swallowed by human beings. An overgrowth of these staphylococci in cream-filled pastries and similar foods has been responsible for a number of outbreaks of acute *food poisoning.*

Disease production by staphylococci. The fact that staphylococci are constantly present on the body surfaces where they usually live quite harmlessly, and yet are able to cause active infection once they pass the barrier of skin or mucous membrane, is sufficient explanation for the great frequency with which these organisms are found associated with inflammations in various parts of the body.

When a cut or abrasion of the skin permits the staphylococci to penetrate, the usual result is the rapid development of a small pustule, or pimple, or a larger abscess commonly called a *boil,* or *furuncle.* This disease condition is called *furunculosis.* The process by which a boil is formed has already been described (p. 313). The rapid accumulation of purulent exudate about the invading staphylococci is the characteristic reaction of the body to the presence of these organisms. Although many other pathogenic bacteria incite pus-formation, this reaction is always marked in the case of staphylococcus infection of any part of the body. For this reason the staphylococci are the outstanding examples of *pyogenic* (pus-forming) *cocci.*

The fact that a furuncle is the usual result of the entrance of staphylococci, indicates that most human beings possess a high degree of resistance against these organisms, and that the cocci are usually of rather low virulence, as compared, for example, with the streptococci. A more severe disease, with invasion of the deeper parts of the body, may occur when the individual is infected with an unusually virulent strain or when the general resistance of the body is abnormally low.

In these conditions, instead of a single localized boil, a considerable area of the deeper tissues of the skin may be invaded with, perhaps, several accumulations of pus reaching to the skin surface. This is called a *carbuncle.*

Osteomyelitis—inflammation of the periosteum and of the soft parts of the bones—is most often caused by staphylococci. This infection may be traced to contamination of a compound fracture wound from without, but commonly it arises from bacteria in the blood stream which localize on a bone that has suffered some kind of mechanical injury. The inflammation may be acute and may soon develop into a septicemia. Often, however, staphylococci of low virulence cause a chronic osteomyelitis which may persist for months or years.

Sometimes the staphylococci reach the blood stream from an original point of infection in considerable numbers, but do not produce a septicemia at once. Instead, the organisms localize in various internal organs, as, for example, the bones, joints, kidneys, lungs, and liver. Wherever they lodge and begin to multiply, an abscess is formed which is essentially the same as a boil. There may be many of these internal foci of infection, all acutely inflamed at the same time, and the patient may be very ill. This is the condition known as *pyemia.*

Any one of these clinical forms of staphylococcus infections may terminate in a fatal *septicemia.* Boils or carbuncles about the nose or lips are especially liable to spread rapidly, the infection extending to the venous sinuses in the membranes covering the brain (dura mater) with consequent clotting of the blood in these sinuses (venous sinus thrombosis). Transfer of infected blood clots from these veins to the lungs and escape of the staphylococci into the general circulation, ends finally in death by "blood poisoning," i.e., by an overwhelming septicemia.

Occasionally, the staphylococci which get into a break in the skin are so virulent, or the patient's resistance is so low, that fatal septicemia develops at once. In young children, a very rapid and severe generalized staphylococcus sepsis is not uncommon, and it is almost invariably fatal.

Staphylococci are usually regarded as the cause of a contagious skin infection occurring most commonly in young children, called *impetigo contagiosum.* The disease is characterized by boil-like pustules on the face or other exposed parts of the body. The sores are spread by scratching. The infection is readily transmitted from one child to another by contact. Unless care is taken, it may spread rapidly among the infants in a hospital nursery.

Staphylococci are also found in a great many other disease conditions. Indeed, they are encountered more constantly than any other kind of organism in inflammatory conditions of various parts of the body. But since their virulence is generally low, they are not often the cause of severe or acute infections other than those already mentioned. They are sometimes associated with *inflammations of the bladder, kidney, or other parts of the genitourinary tract.* They sometimes play a leading rôle in *infections of the middle ear,* in *meningitis,* and in *bronchopneumonia* following measles or influenza. Certain toxigenic strains, as we have explained, are responsible for some cases of acute *food poisoning.*

Bacteriological diagnosis. A smear made directly from the pus of a boil or other staphylococcus lesion will reveal the Gram-positive cocci in their characteristic grouping. A culture upon plain agar will permit differentiation between the *albus* and *aureus* varieties and on blood agar will show the hemolytic strains. In cases of pyemia or septicemia a culture from the patient's blood will contain the staphylococci.

Immunity. Resistance to staphylococci seems to depend upon general bodily vigor and it is not increased permanently by recovery from infection. On the contrary, one attack of furunculosis is often followed shortly by another.

Specific treatment. It has been found helpful, in many cases of furunculosis, *to treat* the patient with an *autogenous vaccine,* that is, a vaccine made up of the staphylococci cultivated from the patient's own lesions. Sometimes such a vaccine brings about a very rapid cure; in other cases it is not effective. The use in treatment of a staphylococcus *toxoid* and of recently developed *antitoxic serums* is still in the experimental stage.

PREVENTION OF SKIN INFECTIONS

It is obvious that serious infections of the skin are less likely to occur in persons who keep the skin clean and who maintain a good state of health.

Nurses, doctors, and laboratory workers should take special precautions to avoid cuts and abrasions of the skin, because they so often must handle materials containing virulent bacteria. Pathogenic streptococci and staphylococci, are likely to be present in the discharges from any wound, in sputum and in much of the other contaminated matter which the nurse is obliged to handle in the course of her duties, and the danger of infection is very real. Whenever the skin is injured a reliable disinfectant, such as tincture of iodine, should be applied at once, and the injured part should be covered with a suitable dressing until it has healed.

REVIEW QUESTIONS—CHAPTER XXVII

1. What kind of bacteria are likely to be found on the normal skin?
2. Name four skin infections caused by fungi.
3. Name and describe the organism associated with acne.
4. Discuss briefly some forms of ulceration of the skin.
5. Name eight bacterial diseases with characteristic lesions of the skin.
6. What is the cause of erysipelas? Describe this infection. What other forms of infection may follow invasion of the skin by streptococci?
7. Name five virus infections that involve the skin. Describe briefly four of these.
8. Name and characterize briefly the principal species of the pathogenic staphylococci. Where are these organisms naturally found?
9. Describe the morphology and staining and the principal physiological properties of staphylococci.
10. What toxic substances may be formed by staphylococci? What is the importance of the so-called *enterotoxin?*
11. What is the commonest form of infection caused by staphylococci? Define *furuncle, furunculosis.* Describe the development of a typical boil.
12. Name and describe briefly other common forms of staphylococcus infection.
13. How may a bacteriological diagnosis of a staphylococcus infection be made?
14. Does a permanent immunity develop after a staphylococcus infection?
15. What method of specific treatment is sometimes successful in furunculosis?
16. Discuss the prevention of skin infections.

CHAPTER XXVIII

INFECTION OF WOUNDS. TETANUS AND GAS GANGRENE

INFECTION OF WOUNDS

BEFORE THE introduction of antiseptic methods by Joseph Lister, whose revolutionary work we have already described in Chapter XX, serious inflammation of operative wounds was regarded as a more or less inevitable feature of surgical practice. But in these days of aseptic methods, infection of a surgical wound from without can result only from carelessness. The elaborate preparations for a modern operation, and the strict aseptic technique practiced in the operating room, in which the attending nurses play so prominent a part, are designed to avoid any possible chance for contamination of the wound. Nevertheless, **surgical wounds** may occasionally become infected because of some break in technique. When this happens, the organisms most likely to be introduced are staphylococci or streptococci. The character and severity of the infection will, of course, vary with the nature and virulence of the germs.

One form of operative wound infection occurs rather frequently. This is a mild inflammation with pus formation about the stitches which close the wounds—the so-called "stitch abscess." This infection is caused by staphylococci of low virulence, belonging to the species *Staphylococcus albus,* which normally inhabit the skin.

Of course, the surgeon must frequently operate in areas already infected. He is at pains to arrange for the adequate drainage of such wounds, so that the accumulated exudate may escape. Usually, when free drainage from a localized focus of infection has been provided for, the defensive mechanisms of the body are enabled to dispose of the remaining bacteria and bring about healing.

Bacillus pyocyaneous infection. The nurse will notice that the pus discharged from some infected wounds, especially deep wounds of long standing, imparts a greenish color to the dressings. This is due to infection with the *Bacillus pyocyaneous* (*Pseudomonas aeurginosa*) the "bacillus of blue-green pus."

This organism is a small, Gram-negative nonspore-bearing, motile rod. It is widely distributed in nature and may be found upon the skin or in the mouth. It has only a slight virulence, but may cause infection of the middle ear, intestinal tract, or other parts, in ill-nourished children, or in adults whose resistance is abnormally low. The bacillus grows readily upon the ordinary culture media. Its characteristic pigment is soluble in water so that the entire medium assumes a green color.

Infection of traumatic wounds. Wounds suffered in warfare and accidental injuries of civil life, in which the flesh is torn open by violence (trauma), are certain to be contaminated by bacteria, and unless such wounds are properly cared for, serious and perhaps fatal infection will follow. We have only to recall the hundreds of persons injured on the streets every day by automobiles to realize that wounds of this type are common. Often these wounds are deep and the tissues are bruised and torn. Bacteria on the skin and the clothing and in dirt and other foreign matter are carried into the depths of the injured flesh. In these wounds conditions are excellent for the growth of pathogenic organisms of *both the aerobic and anaerobic types,* and *bacteria which are ordinarily saprophytic may be able to develop there also,* because of the presence of dead tissue.

Conspicuous among the many kinds of *aerobic* bacteria likely to be present in such wounds are *staphylococci* and *streptococci.* There is always the possibility that these organisms, especially the streptococci, may be so virulent as to cause a severe local inflammation or a fatal septicemia.

The greatest danger in connection with infection of traumatic wounds, however, lies not in the presence of these aerobic bacteria, but in the almost unavoidable contamination of the wound with certain pathogenic *anaerobic spore-bearing bacilli.* Among these virulent anaerobes are the germs which cause *tetanus* and *gas gangrene.*

The tetanus bacillus and most of the other anaerobic bacilli associated with wound infections are *saprophytic organisms,* and it is only when they are accidentally introduced into the tissues at the time of a traumatic injury, that they cause disease. The germs are "opportunists." They are normal inhabitants of the intestine of animals and human beings. They reach the soil through the excreta, and exist there for long periods in the spore state. The spores of these germs are widely distributed, and may be present in street dust and in the soil practically everywhere. It is obvious that they are likely to be carried into any traumatic wound, especially if the injury occurs out-of-doors.

TETANUS

This disease has been known for centuries. It is characterized by abnormal and continuous contractions of the muscles, beginning often in the neighborhood of an infected wound, usually involving all the muscles eventually, and causing death. The frequency with which the muscles of the jaw are involved has given rise to the popular term *lockjaw.*

Kitasato, in 1889, solved the problem of the cause of tetanus. He obtained pure cultures of an anaerobic spore-bearing bacillus from cases of tetanus and with these cultures was able to reproduce the disease in animals.

Clostridium tetani. *Morphology and staining.* The tetanus organism is a slender, Gram-positive bacillus. Young forms are motile. It develops round spores which are larger in diameter than the rod and appear at one end, so that the bacterium looks like a microscopic drumstick (Fig. 64).

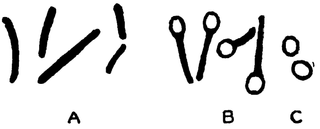

Fig. 64. *Clostridium tetani.* A, vegetative forms; B, the characteristic drum-stick shaped sporulating forms; C, free spores.

Occurrence. The tetanus bacillus is a common inhabitant of the intestine of horses and other herbivorous animals, and also it is frequently present in the human intestine. It is remarkable that this very dangerous organism can exist there without harm. It forms spores when it leaves the body in the feces and is deposited upon the soil. The tetanus spores are extremely resistant. If protected from sunlight or other injurious influences they may remain alive for many years. These resistant spores become widely distributed in the soil and on dirty objects everywhere.

Physiological properties. The tetanus bacillus is an *anaerobe.* It will not grow in the presence of air, unless it is mixed with some of the aerobic bacteria which have a marked avidity for oxygen. When anaerobic conditions are provided, it grows readily on ordinary culture media. It develops best at body temperature.

Products of growth. Tetanus toxin. The one outstanding property of the tetanus bacillus is its capacity to produce a powerful *exotoxin.* It is this poison that accounts entirely for the disease we call tetanus. The toxin is secreted by the living germs as they grow in culture media or in a wound. The poison, like all exotoxins, is soluble in water; thus it appears in solution in the broth in which the organisms are growing, and a clear, sterile filtrate containing the poison can be obtained by passing the culture through a bacteriological filter.

The tetanus toxin is one of the most powerful poisons known. The filtrate of a broth culture may contain enough toxin in one cubic centimeter to kill literally millions of mice. Human beings are even more susceptible to this toxin than mice. It is easy to understand, therefore, how slight a development of the tetanus organisms in a wound may be sufficient to cause a fatal toxemia.

Tetanus in man. *Mode of infection.* Despite the almost universal distribution of tetanus spores, and the frequent occurrence of wounds which must be contaminated with them, tetanus is not a common disease. This is explained by the fact that the tetanus spores cannot germinate and multiply to produce toxin unless conditions in the wound suit their requirements. They are saprophytes and not adapted to invasion of healthy tissue, hence the presence of some dead flesh, which may give them a start, is probably essential. The presence of other organisms in the wound favors the development of the tetanus germs. Further, the tetanus bacilli are anaerobic and will not develop unless carried deep into the tissues, where there is little atmospheric oxygen, or unless accompanied by aerobic organisms that use up the oxygen. Hence the character of the wound and the number and kind of other bacteria present are the most important factors in determining whether or not tetanus is likely to develop. Badly lacerated wounds in which splinters of wood and bits of glass are embedded, gunshot wounds, and wounds made by blank cartridges in which particles of paper or cotton wadding bearing tetanus spores are driven into the flesh, and other types of *penetrating wounds*, are especially liable to furnish the proper conditions for growth of tetanus germs. People justly fear to step upon a rusty nail, though the fact that nail is rusty has little importance, except that, being rusty, it is perhaps more likely to be soiled with earth and it might break off in the puncture wound. Rarely, tetanus has occurred following contamination of a smallpox vaccination sore, or other superficial wound or ulceration, and after surgical operations upon the intestine. The use of suture material (usually catgut) contaminated with tetanus spores probably explains most of these rare cases of postoperative tetanus. In infants, fatal tetanus may follow if the germs enter the cut surface of the umbilical cord.

Action of tetanus toxin. In any case, the tetanus bacilli do not multiply to any extent in the wound, nor do they invade the deeper tissues. The disease is caused by their *toxin* which has a marked affinity for nervous tissues. It has long been taught that this toxin travels from the wound along the motor nerves to the spinal cord, and that symptoms appear when the poison reaches the central nervous system. Recently Abel has shown, however, that the toxin probably does not travel directly along the nerves, but is carried from the site of infection by way of the lymphatics and blood, and is transported to the central nervous system by the blood stream. In severe cases the interval between beginning of the infection and appearance of symptoms (incubation period) is about seven days. In milder cases, which sometimes recover, the incubation period may be as long as a month or more. Tonic contractions of the muscles occur, beginning usually in the region of the wound, and often affecting very early the muscles of the jaw, until in fatal cases all the muscles of the body are involved.

Tetanus antitoxin. *Production and standardization.* Since the poison of the tetanus bacillus is a true exotoxin, it is possible to prepare a specific neutralizing *antitoxin* for it. This is now made commercially by inoculating horses repeatedly with the pure toxin, until a large amount of the specific antibody (antitoxin) has accumulated in the blood of the animals. The purified, concentrated blood serum of these immune animals constitutes tetanus antitoxin as we have it on the market.

The antitoxin is standardized by testing its protective power for guinea pigs inoculated with the toxin. In the Government laboratories of the National Institute of Health in Washington, a standard tetanus antitoxin and an official test dose of tetanus toxin are carefully preserved. A *unit of tetanus antitoxin*, according to the methods used in the United States, is *ten times the least amount of serum necessary to save the life of a 350-gram guinea pig for ninety-six hours when the animal is inoculated subcutaneously with the official test dose of tetanus toxin.* The Government distributes samples of the standard antitoxin to serum manufacturers so that they may standardize their product against it.

Therapeutic use. Once the symptoms of tetanus have fully developed, there is very little hope of recovery, because tetanus antitoxin cannot neutralize toxin which has already combined with the tissues. Nevertheless, the life of the patient is sometimes saved by the intravenous injection of antitoxin, which serves at least to protect the body from further damage. Mild cases, in which only a few groups of muscles are involved, have a good chance of recovery with early antitoxin treatment.

Prevention of tetanus. Prophylactic use of tetanus antitoxin. The great value of tetanus antitoxin lies in its prophylactic use. When an injection of the antitoxin (about 1,500 units) is given a patient within a few hours after the wound is suffered, the chances of developing tetanus are greatly reduced. The value of this measure was demonstrated dramatically during the World War. In the British army about 32 out of every 1,000 wounded men developed tetanus in the first months of 1914, but when the prophylactic use of tetanus antitoxin became general the rate dropped to about 1.5 per 1,000 wounded and remained at about that level throughout the war. It is now the general practice everywhere to administer a prophylactic dose of tetanus antitoxin at the earliest possible moment to *every individual* who has suffered a wound which may lead to tetanus.

It must be remembered that the passive immunity conferred by a single injection of tetanus antitoxin wears off in about two weeks, and when the antitoxin has all left the body, the individual is probably as susceptible to tetanus as a person who has never been inoculated at all. Hence, if tetanus bacilli are still present in the wound, they may yet give rise to the disease. *A second dose* of the antitoxin, about a week later, should be given if the wound is not healing properly. Also in case it should be necessary to operate later in the area of the original wound another dose of the antitoxin should be given. The tetanus spores originally introduced may remain alive but inactive for weeks, ready to germinate if the tissues are again devitalized by surgical procedures.

In addition to the use of antitoxin, there is one other important measure which helps greatly in prevention of tetanus, namely—the *surgical cleaning of the wound.* This consists in the removal of foreign bodies and the cutting away of dead tissue so that the injured flesh is converted, so far as may be possible, into an open, clean wound, in which anaerobic organisms are not likely to thrive.

Active immunization with tetanus toxoid. By adding about 0.3 per cent formaldehyde to tetanus toxin, and holding the mixture at 37° C. for several months the toxin is converted into *toxoid,* in which state it has lost its poisonous properties but retains its antigenic power, i.e., its power to stimulate the formation of a specific antitoxin. Recent trials have shown that two or three injections of this harmless toxoid into human beings results in the appearance, after five or six months' time, of a high concentration of tetanus antitoxin in the blood—a strength of antitoxin amply sufficient to protect against any likely exposure to the risk of tetanus. This protection may be raised still higher, and made more lasting, by a second series of injections of the toxoid about one year later. Persons thus actively immunized who are exposed to tetanus because of a traumatic wound, might be given an injection of toxoid, rather than the usual antitoxic serum, for the toxoid would call forth an almost immediate outpouring of their own tetanus antitoxin into the blood stream which would act as an effective preventive. Substitution of this *active* immunization for the customary anti-tetanus serum injection would be desirable, for it would avoid the frequent and sometimes serious serum reactions, and would reduce the number of individuals who now become sensitized to horse serum following the routine inoculation of prophylactic tetanus antitoxin. Active immunization will probably be utilized to protect soldiers in the future.

GAS GANGRENE

Aside from tetanus, other serious forms of wound infection may develop, following severe traumatic injury, when the wounds are not promptly cleaned or properly dressed. War wounds are naturally more liable to these complications than the accidental injuries of civil life, which are usually well cared for. These infections may take various clinical forms, but all are generally included under the term *gas gangrene*.

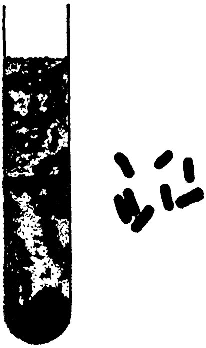

FIG. 65. *Clostridium welchii.* The sketch at the left illustrates the characteristic "stormy fermentation" produced by growth of this organism in a tube of milk medium. The milk is first clotted, then the clot (shown in black) is disrupted by the pressure of the gas formed by the organism.

In typical cases there is an acute, rapidly spreading, destructive infection, involving especially the muscles. The tissues about the wound become greatly swollen, discolored, and infiltrated with gas; often they are crepitant, i.e., one can hear (and feel) the movement of fluid and gas within them. A thin, frothy, bloody fluid, which often has a nauseating odor, and may contain obvious bubbles of gas, exudes from the wound. The pressure of the gas in the tissues is so great that it actually tears them apart. The patient is at the same time poisoned by toxins secreted by the germs. The gangrene extends rapidly and is frequently fatal, even though attempts are made to stop it by amputation of the wounded limb.

The organisms responsible for this frightful condition are anaerobic spore-bearing bacilli of several species. Like the tetanus bacillus, they are primarily saprophytic and are frequent inhabitants of the intestine. Their spores are carried into the wounded flesh with soil, and their development depends, as in tetanus, upon favorable conditions within the wound. Once they begin to multiply they elaborate poisons which so damage the body that they are enabled to invade the tissues rapidly. The most important species of these anaerobes, are *Clostridium welchii*, *Clostridium oedematiens*, and *Clostridium oedematis-maligni* (*Vibrion septique*). Of less importance are *Clostridium sporogenes* and *Clostridium histolyticum.* The latter two species are not themselves capable of causing gas gangrene, but by their intense proteolytic action assist the other organisms in their attack on the tissues. Usually two or more of the anaerobes are present in the same infected wound, together with aerobic bacteria. *Clostridium welchii,* however, is the predominating organism in the majority of typical cases of gas gangrene.

Clostridium welchii. This bacillus is a short, Gram-positive, nonmotile rod (Fig. 65). It is named for Dr. Welch, who, with Nuttall, first described it in 1892. In the tissues it develops a thick capsule. Outside the animal body it forms large oval spores. It is found almost constantly in the intestinal contents of animals and men. It is frequently present in the stools of infants. Like the tetanus organism, its spores become widely distributed in the soil.

It grows readily in laboratory cultures under anaerobic conditions. Its most outstanding property is its extremely vigorous fermentation of sugars, with the production of very large amounts of gas. Hence it is commonly called the "*gas bacillus.*" The rapid fermentation of the lactose in milk gives a characteristic appearance to cultures in this medium, the so-called *stormy fermentation of milk* (Fig. 65). Many strains are able to cause hemolysis of red blood cells.

The disease-producing power of *Clostridium welchii* depends in part upon its very active gas-formation and in part upon the poisons it develops during growth. In wounds, the organisms ferment the muscle sugar, causing the formation of gas bubbles which by their pressure disrupt the tissues, and carry the infection further into the body. The poisons developed by the germs include a true exotoxin. This poison is not so potent as tetanus toxin, but nevertheless, as we have already indicated, plays an important part in the production of gas gangrene. The organisms become numerous in the infected area, but usually do not invade the blood stream except in the few hours before death.

Clostridium welchii is often found associated with pathological conditions other than gas gangrene. It is one of the organisms which may cause *diarrhea in infants.* It is sometimes the cause of fatal blood poisoning, especially in the cases of *septicemia which follow a criminal abortion.* It is also regarded as one of the most important bacteria associated with *appendicitis.*

Antigangrene serum. Antiserum (containing antitoxin and other protective antibodies) prepared against *Clostridium welchii* and also against the other important anaerobic bacilli of gangrenous infections, has been used with some success in the prevention and treatment of gas gangrene.

REVIEW QUESTIONS—CHAPTER XXVIII

1. Under what circumstances are surgical wounds likely to become infected? What is the cause of "stitch abscess"? Why does the surgeon leave drains in infected wounds?
2. Name and describe the "bacillus of blue-green pus."
3. How do traumatic wounds become infected? What two aerobic organisms are likely to be present in infected wounds and what is their importance?
4. Explain how the germs of tetanus and gas gangrene may get into wounds.
5. What characterizes the disease called tetanus? Who discovered the germ and when?
6. Describe briefly (*a*) the morphology and staining, (*b*) the occurrence, and (*c*) the important physiological properties of *Clostridium tetani.*
7. What is the one outstanding property of the tetanus bacillus which accounts for the disease it causes?
8. Describe the types of wounds which are likely to lead to tetanus in man and explain why special circumstances are necessary to permit the development of the organism.
9. How does tetanus toxin act to produce the disease?
10. How is tetanus antitoxin produced and standardized?
11. What value has tetanus antitoxin in the treatment of tetanus?
12. Discuss the use and value of tetanus antitoxin for the prevention of tetanus. What other measure is important?
13. What is tetanus toxoid? Discuss its use and value in the prevention of tetanus.
14. Describe the infection called "gas gangrene." Name the three most important organisms associated with this disease. Name two other bacteria of lesser importance and explain their part in the infection. What species is usually predominant?
15. Briefly describe the properties of *Clostridium welchii.* Why is it called the "gas bacillus"?
16. Explain how this organism brings about gas gangrene. What other pathological conditions may it cause?
17. What is antigangrene serum?

CHAPTER XXIX

INFECTIONS OF THE MOUTH AND THROAT. STREPTOCOCCUS INFECTIONS. SCARLET FEVER. VINCENT'S ANGINA

INFECTIONS OF THE MOUTH AND THROAT

THERE ARE no regions of the body which normally support a more abundant and varied population of microbes than the mouth and throat. The moist, warm mucous membrane furnishes excellent conditions for the life of all types of microbes. Bacteria thrive especially well in remnants of food and in the débris of dead epithelial cells about the teeth. The peculiar anatomy of the mouth cavity and throat affords sheltered living places for numerous types of anaerobic as well as aerobic bacteria. Anaerobic organisms are especially abundant about the gum margins, in crevices between the teeth, and in the deep folds, or crypts, in the surface of the tonsils.

A list of the microbes which have been cultivated at one time or another from the human mouth reads like a catalogue of all the main groups of micro-organisms. Numerous species of cocci, bacilli and spirilla, and also several kinds of higher bacteria, spirochetes, yeasts, and mold-like organisms, as well as amebae and other protozoa, may be present. When blood agar cultures are made from the healthy throat, the most conspicuous *aerobic* organisms are *Gram-negative diplococci* (*Neisseria*) of several species, and *Gram-positive cocci,* including *Staphylococcus albus* and *aureus,* and streptococci of the variety that produces a colony surrounded by a green zone (*Streptococcus viridans*). The hemolytic variety of streptococcus (*Streptococcus hemolyticus*) is not common in the healthy throat. *Pneumococci,* and *diphtheroids,* are almost constantly present.

Many of these organisms found normally in the mouth and throat are entirely harmless. Others, like the streptococci, are potentially pathogenic, and are frequently associated with disease. *Healthy carriers may have in their throats fully virulent diphtheria bacilli, the germs of meningitis, or other dangerous organisms.*

In the mouth of nearly every person there are places where the gums are not anatomically perfect—they do not meet the teeth with a knife edge, but instead, the gum margin is blunted and easily loosened from the teeth. If a needle is passed along this blunted margin or forced between the gum and the tooth and the material thus secured is examined under the microscope, a seething mass of bacteria is revealed (Fig. 66). Conspicuous among these organisms are delicate spiral threads of irregular form. These are spirochetes. Slender spindle-shaped rods, with pointed ends are also numerous. These are called *fusiform bacilli.* Like the spirochetes they are *anaerobes.* In cultures from such material one of the most abundant organisms is a small Gram-negative anaerobic bacillus, called *Bacterium melaninogenicum,* so named because it produces a brownish black melanin-like pigment on blood agar. The number of these bacteria in the mouth depends upon the degree of cleanliness of the teeth as well as upon the anatomical condition of the gum margin. A very simi-

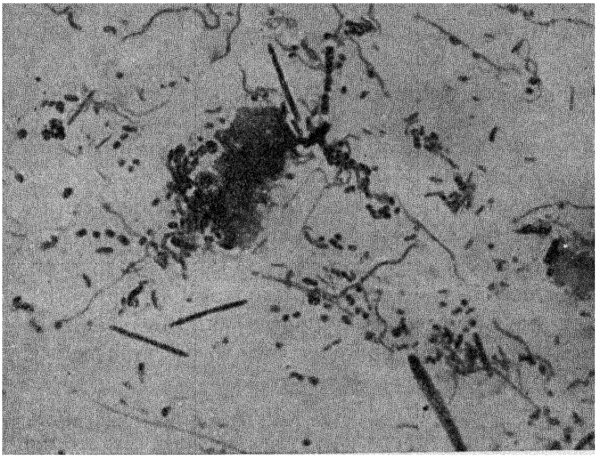

FIG. 66. A direct smear from the margin of the gum in a normal mouth, showing spirochetes, fusiform bacilli, and other organisms.

lar flora is present in the tonsillar crypts. It is important to realize that these very organisms in the normal mouth are often found associated with disease, such as ulcerations in the mouth or throat and abscesses about the head and neck. The origin of lung abscesses, particularly those following an operation in which ether anesthesia was used, may often be traced to bacteria from the mouth, and the anaerobic types mentioned above are usually abundant. These facts make clear the great importance of mouth hygiene.

Diseases of the gums and teeth. Despite this large population of microbes in the mouth, the gums, though frequently injured, are rarely infected. On the other hand, *caries or decay of the teeth*, *abscesses about the teeth*, and *pyorrhoea* are common diseases.

The cause of caries of the teeth is not certainly known. A very prevalent idea is that acid, developed as a result of the growth of bacteria in particles of food caught between the teeth, eats through the outer surface (enamel) of the teeth, thus exposing the softer dentine beneath. As the decay goes on, a hole appears which must be filled by the dentist if the tooth is to be saved. Some authorities do not agree with this idea of the origin of caries, but assign great importance to the influence of faulty diet.

Nearly everyone suffers at some time from an "ulcerated tooth" or a milder form of toothache. These conditions are due to an acute or chronic infection about the root of a tooth or in the root canal of the tooth. Several types of bacteria may be responsible for the inflammation, including staphylococci, and other organisms found normally in the mouth, but probably the majority of cases are due to the *Streptococcus viridans*, the green colony variety of streptococcus described below.

These tooth infections are often regarded as of no consequence, although an inflammation causing so much pain and distress in another part of the body would be considered a very serious matter. As a matter of fact, neglect in proper treatment of an infected tooth may have grave consequences. If the infection becomes chronic, it may serve as a focus from which germs may be carried to internal organs by the blood, and contribute to the development of more serious pathological conditions, such as heart disease. Such *focal infections* about the teeth, in the tonsils, in the middle ear, in the sinuses of the head, must inevitably impair the general health of the individual. They are thought to be among the most important contributing causes of arthritis, rheumatism, and heart disease.

In the condition known as *pyorrhoea alveolaris* there is a chronic inflammation with pus-formation about the roots of the teeth and a slow destruction of the alveolar bone, so that the teeth eventually become loosened. The cause of this rather common disease is unknown. In the pockets of ill-smelling pus about the affected teeth a great many bacteria of different kinds are present, but their relation to the cause of the condition has never been clearly established.

Infections of the mouth, pharynx, and tonsils. *Thrush*, a disease of the mouth sometimes seen in children and caused by a yeast-like organism, has already been mentioned (see Chapters VI and XL). Inflammation of the pharynx (*pharyngitis*, or sore throat) is too familiar to require description. It is not always caused by the same germs. In a majority of acute cases, however, the predominating organisms in a sore throat are *streptococci* of the hemolytic variety. The same is true of the various clinical forms of *tonsillitis*. Streptococci also caused the throat inflammation in *scarlet fever* and the milk-borne disease called *septic sore throat*. These and other streptococcus infections are described in the following sections.

Aside from these conditions, there are two common and important *specific* infections which produce characteristic lesions in the mouth or throat, namely *Vincent's angina* and *diphtheria*. The germ of *syphilis* also sometimes infects the mouth or tonsils, causing lesions which must be distinguished from those of Vincent's angina or diphtheria.

STREPTOCOCCUS INFECTIONS

The pathogenic streptococci. *Species and varieties and their distribution.* Bacteriologists have found it very difficult to classify the many varieties and subvarieties of streptococci which are found in the mouth and throat and in connection with disease. It is not clear which types of these organisms should be regarded as separate species. For practical purposes, however, the streptococci may be grouped *according to their action upon the blood* *in blood agar media.* On this basis three groups of streptococci are generally recognized: (1) the *hemolytic colony* or *beta type* streptococci, whose colonies on blood agar are surrounded by a clear, transparent zone in which the red blood cells are destroyed and there is complete decolorization of the hemoglobin (Fig. 67), (2) the *green colony, viridans* or *alpha type* streptococci, whose colonies are surrounded by a zone in which the red color of the hemoglobin has been changed to a green or brownish green, while the blood cells remain virtually intact, and (3) the *indifferent colony, anhemolytic* or *gamma type* streptococci, whose colonies produce no visible change in the medium. Often colonies of the green (*alpha*) type show at the periphery a narrow transparent zone of partial "hemolysis," in which there is a more or less complete decolorization and destruction of red blood cells, but the term "hemolytic streptococci" is by custom applied only to organisms of the first-mentioned, *beta* type.

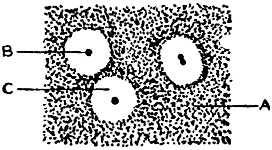

FIG. 67. Appearance of colonies of *Streptococcus hemolyticus* on blood agar. A, the opaque medium; B, the colony; C, the clear transparent zone about the colony, in which "hemolysis" (destruction and decolorization) of the red blood cells in the medium has occurred.

The name *Streptococcus hemolyticus* is generally applied to the *hemolytic streptococci.* Included in this group are the most virulent strains, and by far the largest number of serious streptococcus infections are due to hemolytic streptococci. They may be found in the perfectly healthy throat, but usually are not encountered except in connection with some pathological condition.

The hemolytic streptococci associated with scarlet fever, erysipelas, and septic sore throat are called, respectively, *Streptococcus scarlatinae, Streptococcus erysipelatis,* and *Streptococcus epidemicus.* It is not certain, however, whether each of these deserves to be considered a separate species.

The *green colony streptococci* are named *Streptococcus viridans.* Many strains are included in this group which differ considerably from each other. They are almost constantly present in the healthy mouth and throat. Most strains are pathogenic, but their virulence is low, as compared with the hemolytic varieties.

The *indifferent colony streptococci* are generally regarded as of little importance. They are frequent inhabitants of the healthy throat.

Morphology and staining. The individual streptococci are tiny spherical bodies, usually less than one micron in diameter. They are arranged characteristically in chains, like a string of beads. Often the chain is made up of pairs of cocci very close together, separated by a considerable space from adjacent pairs, so that the chain appears as a series of diplococci end to end. The length of the chain varies with the conditions of growth, and also to some extent with the variety of streptococcus. Virulent hemolytic streptococci may develop a capsule.

All the pathogenic streptococci are Gram-positive.

Physiological properties. The streptococci do not grow as readily on artificial culture media as the staphylococci, but will develop well on media enriched with blood or blood serum. Even on these media, however, their growth is comparatively delicate, and their colonies small. They multiply best at body temperature. Most of the streptococci are aerobic; there are a few strictly anaerobic varieties.

The streptococci die off quickly in laboratory cultures and in general have little resistance outside of the body. *They may remain alive in pus, however, or in sputum or similar materials for several hours.* They multiply readily in milk, and milk may serve as the vehicle by which the germs are spread, as in epidemics of septic sore throat and sometimes in scarlet fever outbreaks.

Products of growth. Toxins. Virulent streptococci, like staphylococci, form a number of products which enhance their disease-producing power. The *hemolysin* (substance that causes destruction of red blood cells) of the hemolytic streptococci is definitely associated with virulence. Most pathogenic strains also produce *fibrinolysin*, which dissolves human fibrin, and *leukocidin* which destroys leukocytes.

Moreover, the strains associated with scarlet fever and erysipelas, at least, and probably other strains, undoubtedly manufacture true *exotoxins* which are poisonous to the body as a whole. These toxins are not so powerful as those of the diphtheria or tetanus germs, but nevertheless they are sufficiently potent to weaken the patient considerably and aggravate the severity of the infection.

Disease production by streptococci. The *hemolytic streptococci* are associated with infections both of mild and of serious character. Streptococci are the organisms most to be feared as the cause of infection of surgical or accidental *wounds*, whether superficial or deep, because of all the common bacteria they are the most liable to invade the deeper tissues to produce a grave *cellulitis*, or severe *osteomyelitis*, and all streptococcus infections tend to terminate in a fatal *septicemia.* Many instances are known in which surgeons or pathologists have developed septicemia as a result of pricking their fingers during an operation or an autopsy on a streptococcus case. At least one member of practically every class of nurses develops a serious streptococcus finger infection sometime during the period in Training School. Extraordinary care must be exercised in handling pus or other material containing virulent streptococci.

Hemolytic streptococci are the frequent cause of acute *otitis media* (inflammation of the middle ear). This inflammation may extend to the mastoid region *(mastoiditis)* and sometimes leads to a fatal *meningitis.*

Streptococci cause most cases of *acute sore throat*, *tonsillitis*, and *peritonsillar abscess.* The variety called *Streptococcus epidemicus* causes the milk-borne epidemic infection, *septic sore throat.* The severe, and often fatal, *bronchopneumonia* which commonly follows an attack of measles or influenza or other weakening disease is often caused by streptococci.

Infection of the uterus and blood after childbirth *(puerperal fever)* is most often due to streptococci introduced from without. The patient usually dies with a septicemia.

Erysipelas is always a hemolytic streptococcus infection and is probably caused by the special variety called *Streptococcus erysipelatis.* An *antitoxin* has been prepared which will neutralize the toxin of this germ and may be used in the treatment of erysipelas.

Scarlet fever is discussed below.

The *green colony streptococci* are more commonly found in less severe infections. They are frequently associated with *tooth abscesses*, and may be present in *otitis media*, or *infections of the accessory sinuses* of the nose, or of the *appendix*, or *gall bladder.*

The *Streptococcus viridans* is often the cause of *subacute bacterial endocarditis*—a form of heart disease in which so-called *vegetations,* consisting of an exudate in which there are masses of streptococci, accumulate on the valves of the heart. The organisms escape intermittently into the general circulation, so that cultures from the blood at these times will contain the organisms, but usually a true septicemia does not develop until just before death, which always occurs in a few weeks or months.

The green colony streptococci and also the indifferent colony variety have been suggested as the cause of *acute rheumatic fever. Streptococcus viridans* has been isolated from the blood of many patients with rheumatism. Cultures from the inflamed joints, however, are usually sterile. Some investigators believe that a particular kind of streptococcus, growing in the throat or elsewhere, produces a poison which causes the disease. Others believe that the acute inflammation of the joints and other symptoms of rheumatism are manifestations of a condition of hypersensitiveness to streptococcus protein. There is some evidence that rheumatism may possibly be caused by a filtrable virus.

Bacteriological diagnosis. Smears and cultures made directly from the inflamed throat or other part, or from the discharges from the lesions, usually show numerous Gram-positive cocci in short or long chains, and there is little difficulty in making the diagnosis. Cultures of the blood will contain the streptococci in cases of bacteremia and septicemia. The growth on blood agar plates will determine whether the streptococcus is of the indifferent, green, or hemolytic colony type.

In case green colonies appear, it is necessary to make sure the organism is not a *pneumococcus,* which develops on blood media a green colony indistinguishable in appearance from that of the *Streptococcus viridans.* Aside from the fact that pneumococci are usually larger cocci in pairs, rather than in chains, the most important differences are: pneumococci ferment inulin, streptococci usually do not; pneumococci are *soluble in bile,* streptococci are *not.*

Immunity. Use of vaccines and serums. Sulfanilamide. Except in the case of scarlet fever, recovery from streptococcus infections does not lead to a lasting immunity. Even after scarlet fever, the resistance of the recovered patient is effective only toward the *toxin* of the scarlet fever streptococci, and protects little, if at all, against the organisms themselves.

Vaccines are not widely used in treatment, though some workers have reported favorable results. Immune serums, prepared by injecting horses repeatedly with cultures of streptococci, have been used with variable results in treatment of various forms of streptococcus infection.

An *antitoxin for scarlet fever*, of great value in treatment of this disease, is available. For the prevention of scarlet fever, a method of *active immunization against the scarlet fever streptococcus toxin* has been developed, as described below.

It is in the treatment of streptococcus infections that the newly-developed chemical remedy *sulfanilamide* (and its derivatives) has had such remarkable success. The chemical is believed to aid the natural body defenses indirectly by making the streptococci more susceptible to phagocytosis.

Prevention. The student will realize that the most virulent streptococci are those coming immediately from an infected individual and that it is by direct contact through the hands or by droplet infection that the organisms are spread. In the hospital the nurse must take especial care to avoid cuts and abrasions of the skin of the hands. The wearing of gauze masks over the nose and mouth is a useful protection against droplet infection and may prevent the nurse from becoming a carrier of virulent streptococci which she might convey to other patients. In view of the great danger of streptococcus infection of the uterus after childbirth, a nurse or other attendant who has just been in contact with a case of streptococcus infection, should not be permitted to go directly to the care of an obstetric case.

SCARLET FEVER

Present conception of the cause of scarlet fever and the nature of immunity to the disease. Although certain Russian investigators really demonstrated the truth of the matter as early as 1907, and many other workers contributed important evidence, it was principally the work of Dick and Dick, beginning in 1923, which finally made clear the true rôle of streptococci in scarlet fever. It is now generally agreed that the streptococci associated with scarlet fever fall into a fairly definite group and it is justifiable to regard them as a separate variety of the hemolytic streptococci, under the name *Streptococcus scarlatinae.* It has been definitely established that these organisms produce an *exotoxin*, which, when absorbed into the blood, causes the fever and other toxic symptoms and the skin rash characteristic of scarlet fever. While the germs usually remain localized in the throat, the poison they secrete is carried throughout the body by the circulating blood. Scarlet fever is much like diphtheria, because in this disease, also, the germ causes a local throat inflammation while its toxin poisons the whole body.

The scarlet fever streptococcus toxin, however, is not so destructive as the poison of the diphtheria bacillus, and by no means so powerful as the tetanus toxin, so that the direct effect of the poison on the body is comparatively unimportant. The greatest danger arises from the tendency of the streptococci themselves to spread from the throat to set up serious inflammation in the middle ear, neck glands, or elsewhere. When death occurs there is a streptococcus septicemia.

Persons who recover from scarlet fever have in their blood an *antitoxin* which specifically neutralizes the scarlet fever toxin. So long as this antitoxin is present, the individual remains immune to the disease. It is very important to remember, however, that although such persons are resistant to scarlet fever *toxin*, and so are not likely again to develop the typical disease, they have *no lasting immunity to the streptococci themselves*, and may later be reinfected with scarlet fever streptococci. When so reinfected, such an individual may transmit typical scarlet fever to susceptible individuals. It follows, then, that the same streptococci may produce in some persons merely a local throat inflammation of more or less severity, and in others a typical scarlet fever, depending upon whether or not the individual has scarlet fever antitoxin in his blood.

How scarlet fever spreads. The germs of scarlet fever are present in the nose and throat discharges and in the pus from infected ears, glands, etc. They are usually transmitted by contact directly from person to person, or more rarely, through contaminated *unpasteurized* milk. The streptococci are present in the nose and throat from the very beginning of symptoms, throughout the disease, and *for weeks after convalescence.* Most health departments require the quarantine of scarlet fever patients in uncomplicated cases for at least four weeks. Even after this long period, the individual may still be able to transmit the disease to others. If there are complications in the form of infected ears or other local inflammations, the patient must be isolated until these have healed and all abnormal discharges have ceased. The "scales" from the peeling skin have nothing to do with the spread of the infection.

The Dick test for susceptibility to scarlet fever. Susceptibility to scarlet fever may be determined by the injection, *intradermally* (just beneath the skin surface), of a small amount of diluted scarlet fever streptococcus *toxin.* The test, called the *Dick test,* after the workers who devised it, is the same in method and principle as the Schick test used for determining susceptibility to diphtheria toxin. The test consists in the injection, into the skin of the forearm, of 0.1 c.c. of an especially prepared toxin solution. The amount of poison in this injection is one "skin test dose," by which is meant, the *smallest amount of the toxin which will give a typical reaction in fully susceptible individuals.*

If the individual is *susceptible, i.e.,* does not have scarlet fever antitoxin in his blood—a characteristic red area of inflammation develops about the test injection, reaching its height in about twenty-four hours and often disappearing within thirty-six hours. This *positive reaction* is due to the direct action of the poison on the cells of the skin. If the individual is *immune—i.e.,* if he has scarlet fever antitoxin in his blood—this antitoxin neutralizes the injected poison, so that there is no change at the site of injection and the test is *negative.*

The results of Dick tests upon many individuals have indicated that the majority of young children, especially those below the age of five, are susceptible to scarlet fever, whereas most adults are immune. This is in accordance with the fact that the greatest number of cases occur in young children.

Scarlet fever antitoxin. Antitoxic serum for the treatment of scarlet fever has been prepared commercially by immunizing horses with the toxin alone or with whole cultures containing the organisms as well as the poison. The strength of the antitoxin is determined by the number of skin test doses of the toxin it will neutralize. The *unit of antitoxin is taken as the least amount which will neutralize fifty skin test doses of scarlet fever streptococcus toxin.*

When this antitoxin is injected early in the course of severe scarlet fever, the patient's condition is usually remarkably improved within a few hours. The antitoxin has little direct effect upon the streptococci in the throat, but relief from the poison seems to give the body increased power to battle with the germs, so that complicating infections of the middle ear, etc., are usually avoided. Good results may also be obtained by the use of blood from persons convalescent from the disease, or from others who give a negative Dick test, in place of commercial horse serum antitoxin.

If scarlet fever antitoxin is injected intradermally into a reddened area of the patient's skin, there will occur a definite and permanent *blanching* of the skin rash at that point within a few hours. This so-called *Schultz-Charlton reaction* is a useful diagnostic test, for the blanching will occur only if the patient actually has scarlet fever.

Prevention of scarlet fever. Active immunization. The occurrence of many mild cases, never recognized as scarlet fever, and the existence of healthy carriers of scarlet fever streptococci, make it very difficult to control this disease. The most strict and careful isolation technique is required to prevent the spread of infection, and the isolation must be prolonged until the germs have been eliminated. Early diagnosis and the prompt use of antitoxin will greatly reduce the severity of the disease.

Nurses and others who are obliged to come in contact with scarlet fever patients are especially liable to be infected, and serious efforts have been made to develop for such persons successful methods of active immunization. Nurses in most infectious disease hospitals are now given some form of vaccination, if Dick tests show that they are susceptible. The method of immunization most widely adopted consists in the subcutaneous injection of three or more doses of diluted scarlet fever streptococcus *toxin.* In a short period after these injections the Dick test should become negative, indicating that immunity to the toxin has been established. The duration of this immunity is uncertain, but it probably lasts for several years.

There are several factors that seriously limit the practical usefulness of this immunization procedure. The inoculation of unaltered toxin often results in severe local or general reactions. The necessity of giving so many injections, with perhaps, loss of time from duty after each dose, is an unfortunate feature. And moreover, the vaccination immunizes only against the scarlet fever toxin, and does not directly increase resistance toward the streptococci themselves. Nevertheless, the practice of testing all members of the nursing staff and other attendants by the Dick test, followed by the active immunization of the susceptible persons with the toxin, has resulted in the almost complete elimination of typical scarlet fever among the personnel of many hospitals.

VINCENT'S ANGINA AND RELATED INFECTIONS

Clinical features of Vincent's infections. The condition known as *Vincent's angina* is an acute infection of the tonsil, or neighboring parts of the throat, characterized by the appearance of a pseudomembranous inflammation followed by ulceration. The same infection may be localized on the gums (Vincent's gingivitis), or in the mouth (Vincent's stomatitis). The disease may occur in varying degrees of severity. In the most typical cases there develops a deep ulcer covered with a foul smelling, grayish yellow exudate on one tonsil only. In some cases, however, particularly in children, the infection may be very extensive, with numerous deep ulcerations over the whole mouth and throat.

The patient is usually not severely ill, and aside from some malaise and difficulty in swallowing, may have no symptoms. The infection usually yields to local treatment within a few days, though persistent and recurrent cases are not uncommon. In most instances the most effective treatment has been found to be *salvarsan,* the same drug as is used in the treatment of syphilis.

The typical Vincent's angina, with ulceration of the tonsil, is a comparatively rare disease. But mild cases of gingivitis, with swollen, tender, or bleeding gums, and with or without the formation of a definite membrane or ulcer, are frequent, and this form of Vincent's infection is probably much more common than is generally realized. During the War many soldiers suffered from a disease called "trench mouth." This was a Vincent's stomatitis and gingivitis with ulcerations on the cheeks and gums.

Vincent's angina is not ordinarily communicable. The occasional cases which occur among the general population usually cannot be traced to any preexisting case, and they do not give rise to new cases. But under unusual conditions, such as may prevail among soldiers in the field or in camp, the infection may apparently become transmissible, and many of the men in the same group may have the disease at the same time. Occasionally outbreaks have been reported among college students.

A very striking feature of the disease, as it is seen in the civil population, is its frequent association with a state of lowered resistance on the part of the patient. The typical tonsillar ulceration occurs often in persons whose resistance has been lowered by other diseases, such as scarlet fever, measles, tuberculosis, diabetes, mercurial poisoning, scurvy, and especially *leukemia* and other blood disorders.

Micro-organisms associated with Vincent's infections. Plaut (1894) and Vincent (1898) first called attention to the constant appearance of certain peculiar micro-organisms in the lesions of the infection since called Vincent's angina. These organisms are of two kinds: (1) so-called *fusiform bacilli*—spindle-shaped or cigar-shaped rods with a thick center and pointed ends, often appearing in pairs, and (2) *spirochetes*—pale-staining coiled threads with shallow and irregular curves. When a smear is made directly from the lesion early in the course of the infection, almost no other bacteria are to be seen. There are very few pus cells (Fig. 68). The same striking picture is found in smears made somewhat later, at the time of deep ulceration, except that at this time there are likely to be a considerable number of other organisms present.

We pointed out in the earlier part of this chapter that fusiform bacilli and spirochetes of similar appearance are to be found in healthy persons about the gum margins and tonsillar crypts. Many authors have suggested that Vincent's angina may be caused by these organisms normally present when for some reason the resistance of the individual is lowered to such a degree that they become invasive.

In histological sections of the ulcerating tissues the spirochetes appear to be especially numerous at the edge of the lesion and seem to be advancing ahead of the bacilli into the healthy tissue. This fact, and also the effectiveness of treatment with salvarsan, which presumably acts upon the spirochete (as it does in syphilis on the syphilis spirochete), suggests that it is the spirochete which has the chief rôle in causing the destruction of tissue and the advancement of the lesion, though possibly not in initiating the infection.

Both the fusiform bacilli and spirochetes are *anaerobic* organisms, and both are difficult to cultivate and study.

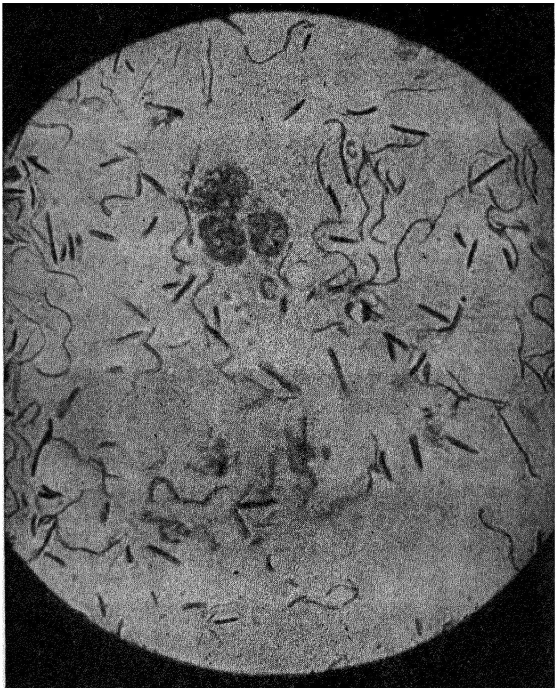

FIG. 68. Direct smear from the ulcerating lesion of Vincent's angina, stained with gentian violet. A positive diagnosis of this disease is made when this characteristic mixture of fusiform bacilli and spirochetes is seen in a direct smear. The possibility that the infection may be complicated by the presence of the germs of diphtheria or syphilis should be considered.

The *spirochetes* have never been satisfactorily classified and it is doubtful whether they have ever been cultivated in pure culture. They are generally called *Treponema vincenti.* As compared with most other spirochetes, they are rather thick and coarse, and have very irregular convolutions. They are highly flexible and have a snake-like motion. They can be colored, though faintly, with the ordinary stains, such as carbol fuchsin or gentian violet.

The *fusiform bacilli* are nonspore-bearing, Gram-negative rods, with ends which taper into a more or less sharp point. They can be cultivated on blood agar under anaerobic conditions. They show no distinctive chemical activities, and do not appear to be pathogenic for animals, at least when in pure culture. There are probably several varieties of fusiform bacilli, but they have not been described with sufficient completeness to warrant recognition as separate species. The name most commonly applied to the best known variety is *Fusiformis dentium.*

Bacteriological diagnosis. The laboratory diagnosis of Vincent's infection depends entirely upon finding of the typical mixture of many fusiform bacilli and spirochetes in *direct smears* from the lesions. These smears are best stained with a strong stain, preferably gentian violet, or carbol fuchsin, in order to be sure that the pale-staining spirochetes will not be overlooked. At present no attempt is made to cultivate the organisms or to identify them further than by their appearance under the microscope.

Differential diagnosis between Vincent's angina, diphtheria, and syphilis. The pseudomembrane which forms in the early stages of a simple Vincent's angina cannot be distinguished by inspection alone from the lesion of diphtheria, and the ulceration which later develops resembles the ulcer which may be caused by the syphilis germ. Furthermore, a Vincent's infection may be *superimposed* as a complication upon either a primary diphtheritic or a primary syphilitic infection. In such cases, a direct smear from the lesion stained by gentian violet or carbol fuchsin would not reveal the syphilis spirochete, if present, because it would not stain, and if diphtheria bacilli were present they would probably be overlooked. Hence, unless other tests are made, the infection might be diagnosed as a simple Vincent's angina and the patient would not receive the necessary treatment and care. Deaths from diphtheria have occurred in persons denied antitoxin because their infection was thought to be an uncomplicated Vincent's angina.

The possibility of confusion in diagnosis between these diseases requires that careful laboratory tests be carried out whenever a patient presents a pseudomembranous or ulcerating lesion in the throat. In addition to direct smears to determine the presence or absence of Vincent's organisms, cultures should always be made on Loeffler's serum medium and examined for diphtheria bacilli. Blood may be secured for a Wassermann test and the likelihood of syphilitic infection must be considered.

Other infections in which fusiform bacilli and spirochetes are abundant. Fusiform bacilli and spirochetes of the same appearance as those in the typical Vincent's infections of the throat and mouth are frequently associated with abscesses, ulcerations, and gangrenous processes in other parts of the body. In these instances, however, their relation to the cause of the condition is doubtful, for they are usually mixed with many other organisms. They probably enter the tissues, which have in some way become vulnerable to their attack, from their normal location on the gum margins and tonsillar crypts, or on the genitals. They appear to play an especially prominent part in many cases of lung abscess and bronchiectasis, brain abscess, and ulcerative and gangrenous lesions about the genitals. They are also abundant in pyorrhoea, and sometimes in conjunctivitis, otitis media, and appendicitis. Sores on the hands caused by human bites are frequently infected with these microbes.

REVIEW QUESTIONS—CHAPTER XXIX

1. Why do the mouth and throat offer particularly good conditions for the growth of micro-organisms? What aerobic organisms are usually found in cultures from the normal mouth and throat? Mention some pathogenic organisms that may be present.
2. Describe the bacteria usually found on the gum margins and in the tonsillar crypts.
3. Discuss the bacteriology of (1) caries of the teeth, (2) tooth abscesses, (3) pyorrhoea.
4. Name a common mouth infection caused by a yeast-like organism. What germ is most often the cause of pharyngitis and tonsillitis? Name two other forms of throat infection caused by the same type of organism. Name three important specific infections of the throat and mouth.
5. What three groups of streptococci are recognized? On what basis?
6. Name the principal "species" of pathogenic streptococci and state where they are usually encountered.
7. Describe the morphology and staining, and principal physiological properties of streptococci.
8. What action may streptococci have upon blood? Discuss toxin pro-

duction by streptococci. Which kinds are known to develop true exotoxins?

9. Name and describe briefly at least twelve forms of infection which may be caused by hemolytic streptococci.
10. Name four types of *Streptococcus viridans* infection. Describe briefly the form of heart disease often caused by this organism. What is a possible relation of streptococci to the cause of rheumatism?
11. How may a bacteriological diagnosis of streptococcus infection be made? How is *Pneumococcus* to be distinguished from *Streptococcus viridans?*
12. Discuss immunity to streptococcus infection. What is the value of streptococcus vaccines and immune serums? What streptococcus infection is successfully treated by an antitoxin and successfully prevented by active immunization? What drug is now widely used in treatment?
13. Discuss the prevention of streptococcus infections.
14. Explain the present day conception of the cause of scarlet fever and the nature of immunity to the disease.
15. How does scarlet fever spread? Why is a long period of quarantine necessary?
16. Explain the purpose and technique of the Dick test. Exactly what is the origin of the material injected?
17. What types of reaction may occur in the Dick test and what does each mean with respect to immunity to scarlet fever? What do the results of many Dick tests show as to the resistance of the general population to scarlet fever?
18. Explain the (*a*) preparation and (*b*) standardization of scarlet fever antitoxin. What is a unit of this antitoxin? What is its value in treating scarlet fever? What other serum may be used in place of commercial antitoxin?
19. Discuss the prevention of scarlet fever. Explain the method of active immunization and discuss its value.
20. Describe the clinical features of typical Vincent's angina, gingivitis, and stomatitis. Is Vincent's angina communicable? Illustrate the apparent relation of "lowered resistance" to the occurrence of the infection.
21. Describe the organisms found in a direct smear from the lesion of Vincent's angina. Discuss their probable relation to the origin and progress of the infection.
22. Describe briefly the spirochetes of Vincent's infection. How are they named?
23. Describe fusiform bacilli. How are they named?
24. Explain how a bacteriological diagnosis of Vincent's infection is made.
25. What two infections may be confused with, or complicated by, Vincent's angina? State clearly what steps are necessary to make a correct diagnosis.
26. Name other infections in which fusiform bacilli and spirochetes are abundant.

CHAPTER XXX

DIPHTHERIA

THIRTY OR forty years ago diphtheria was among the most dreaded of all diseases. But now we have an almost complete mastery of it. We know its cause *(Corynebacterium diphtheriae)*, we have a specific remedy (diphtheria antitoxin), a means of determining susceptibility (Schick test), and successful methods for making individuals immune to the disease (active immunization with toxin-antitoxin mixtures or with toxoid).

The clinical disease. The disease of diphtheria is due partly to the local disturbance in the throat or other parts of the upper respiratory tract caused by the growth of the germs themselves in these places, and partly to the poisoning of vital internal organs by a powerful *exotoxin* secreted by the organisms. The illness begins after an *incubation period of two to five days.* The germs rarely penetrate deeply into the body; their growth is ordinarily confined to the superficial layers of the mucous membrane—usually to a small area of the throat or nose—where they cause a peculiar kind of inflammation characterized by the formation of a tough, grayish white *pseudomembrane.* The leathery character of the membrane gave origin to the name *diphtheria*, which comes from the Greek word meaning *leather.*

In the days before antitoxin was widely used, severe cases of diphtheria were common in which this membranous inflammation extended over the whole surface of the throat and down into the larynx and even into the trachea below. This caused the condition known as "membranous croup." Frequently the membrane formation in the air passages caused death by suffocation. Now this severe form of diphtheria is rare in adults, but still occurs occasionally in children. It is often necessary in these cases to make an opening into the windpipe through the neck (tracheotomy)—or to place a tube in the larynx in order to assure a passage for air.

Prompt diagnosis and early use of antitoxin have almost banished these grave forms of diphtheria and now in most cases the local inflammation in the throat is not in itself a serious matter, and, indeed, in some instances there may be no visible membrane at all. *The chief damage is done by the poison absorbed from the growing germs.* This toxin, carried about by the blood, injures important tissues, especially certain nerves, and the heart muscle. In some cases not effectively treated with antitoxin, paralysis of various muscles may develop a month or more after the acute illness has subsided.

Diphtheria, then, is essentially a *toxemia*, and *resistance to the disease depends upon the presence of diphtheria antitoxin in the blood.*

Corynebacterium diphtheriae. The germ of diphtheria was first described by Klebs in 1883, but it was Loeffler, in 1884, who isolated the organism in pure culture and later proved it to be the sole cause of the disease.

Morphology and staining. The *Corynebacterium diphtheriae* is one of the very few pathogenic bacteria that can be recognized with some degree of certainty merely by its appearance under the microscope. The organisms are slender rods, often slightly curved, showing a characteristic irregularity, both in form and in staining. They tend to lie in groups, in which all the organisms are parallel or nearly parallel. With regard to staining, three types may be recognized: (1) the granular forms, in which deeply colored metachromatic granules appear at the ends or near the center of the rods, (2) the "barred forms" which seem to be crossed by bands or bars of material which does not stain, and (3) even-staining forms (Fig. 69).

These various forms of the diphtheria bacillus may occur in the same culture, and there is no proof that any particular form is more virulent than another.

The characteristic irregularity in staining is best brought out when smears are colored with Loeffler's methylene blue, toluidine blue, or some one of the special stains designed to demonstrate metachromatic granules. The bacilli are Gram-positive. They have no spores or capsules, and are nonmotile.

Physiological properties. Diphtheria bacilli grow best in the presence of abundant oxygen and at body temperature. They will live even on plain agar and broth, but they develop more luxuriantly on medium enriched with blood or blood-serum. The most widely used medium for diphtheria bacilli is the one devised originally by Loeffler, and called *Loeffler's serum medium.* This is made by mixing three parts of beef serum with one part of a 1 per cent dextrose infusion broth. The mixture is coagulated by heat in tubes held in a slanting position, so as to make a solid medium in the form of slants. It happens that on these Loeffler's medium slants diphtheria bacilli grow exceedingly well. In the first eighteen hours of incubation they will grow faster than nearly all the other organisms likely to be present in cultures from the throat or nose, hence this medium is especially well adapted for use in the diagnosis of diphtheria and the detection of carriers of the germ.

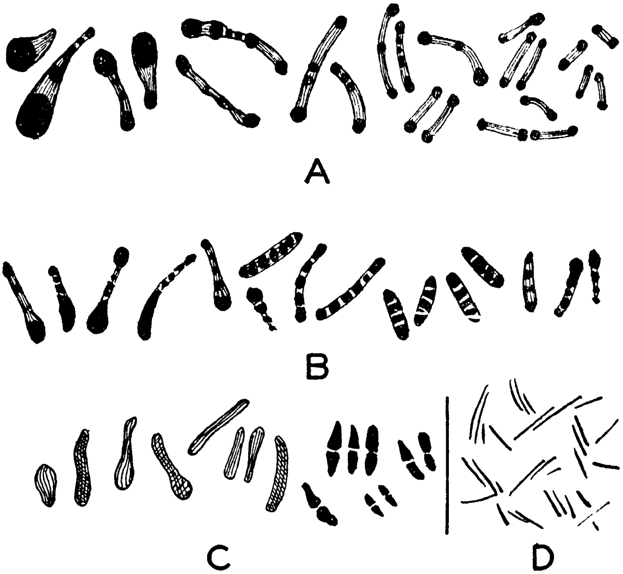

FIG. 69. *Corynebacterium diphtheriae.* A, granular forms; B, barred forms; C, even-staining forms. D, sketch to show the characteristic parallel arrangement of the bacilli in a smear.

Since the diphtheria bacilli have no spores, they are killed rapidly by boiling or by the usual chemical disinfectants. They have more resistance to drying, however, than most other germs and *may remain alive for many weeks on dried bits of the pseudomembrane* from an infected throat.

Diphtheria toxin. The most important property of the diphtheria bacillus is its capacity to form the powerful *exotoxin* which is responsible for the toxemia in diphtheria. When virulent organisms are grown for several days in a suitable liquid medium, a large amount of this peculiar poison is developed. By passing such a culture through a bacteriological filter, a clear sterile filtrate is secured which contains the toxin in solution. The poison in this filtrate can be concentrated somewhat, and has been shown to be protein-like. But the purified toxin is not generally available, and ordinarily, when we say "diphtheria toxin," we are referring to nothing more than a sterile filtrate containing the toxin obtained as indicated above (Fig. 60).

Diphtheria toxin is very poisonous to horses, dogs, cats, rabbits, and guinea pigs as well as to man. To measure the strength of the poison subcutaneous injections are made into guinea pigs to determine the *smallest amount, which, on the average, will kill a 250-gram guinea pig in four to five days.* This amount is called the *minimum lethal dose* (M.L.D.). Most virulent diphtheria bacilli will yield a toxin so strong that as little as 0.002 c.c. contains one M.L.D. for a guinea pig.

It is important to remember that not all strains of diphtheria bacilli produce toxin. Such *nonvirulent strains* are often found in the throats of carriers.

Toxoid. Diphtheria toxin is very unstable, and changes when exposed to air, light, moderate heat, and certain chemicals into what is called *toxoid.* In this state it is *nonpoisonous,* but *still capable of combining with antitoxin* and *will stimulate the formation of diphtheria antitoxin* when injected into the body.

A form of *diphtheria toxoid*, called *anatoxin* by Ramon who first devised it, is now in wide use for the active immunization of horses for the preparation of commercial diphtheria antitoxin, and also for the immunization of human beings. The toxoid is made in the same way that tetanus and staphylococcus toxoids are prepared. About 0.4 per cent of formaldehyde is added to diphtheria toxin and the mixture is allowed to stand until it loses its poisonous properties.

How diphtheria spreads. The germs of diphtheria are conveyed from person to person by way of the fingers, or articles, such as eating utensils, toys, pencils, or handkerchiefs, contaminated with nasal discharges and saliva, or through droplets expelled from the throat and nose during sneezing or coughing. The disease spreads most rapidly among school children and persons in crowded places. In diphtheria, as also scarlet fever, typhoid, and other diseases, *mild, unrecognized cases* of the infection and *healthy carriers* of virulent germs are the commonest sources of new cases. Occasionally epidemics of diphtheria have been traced to contaminated raw *milk.*

Carriers of diphtheria bacilli are numerous, especially in groups of people who live in close quarters, as in tenements, boarding schools and other institutions, army camps, and the like, and among hospital attendants, nurses, and doctors who come in frequent contact with cases of the disease. The bacilli tend to remain in the throat for several weeks after recovery from diphtheria, and some persons become chronic carriers and harbor the germs for months or years. Individuals who have never had any symptoms of diphtheria also may carry the germs. Sometimes the organisms thus carried are not toxin producers, *i.e.*, nonvirulent, and therefore are not dangerous to others, but often fully virulent bacilli are carried for months by individuals who are themselves immune, though constituting a source of infection for other (susceptible) persons. It is sometimes extremely difficult to get rid of the germs in the throats of these chronic carriers. But when pathological conditions of the nose and throat, such as deviated nasal septum or inflamed tonsils, are corrected, the diphtheria bacilli usually disappear.

Bacteriological diagnosis and detection of carriers. While the final decision as to whether or not a patient has diphtheria, and needs antitoxin, must rest with the physician, laboratory tests are of great aid in the diagnosis of this disease. Bacteriological diagnosis depends upon the finding of virulent diphtheria bacilli in cultures from the nose and throat. Carriers are detected by the same means. These cultures can be made so easily, and they give such reliable information, that *they should never be omitted in any case of sore throat which might possibly be diphtheria.* It should be remembered that there are cases of diphtheritic infection of the throat without fever or other obvious symptoms, and without membrane formation. Further, membranous inflammations resembling the lesion of diphtheria may be caused by other organisms, such as streptococci and the germs of Vincent's angina. In these clinically doubtful cases a definite diagnosis must depend upon laboratory cultures. It is exceedingly important that diagnosis of diphtheria be made as *early as possible*, so that the patient will have the benefit of receiving antitoxin before the poison has done serious damage to the body.

Presumptive diagnosis. Two steps are required to make a presumptive bacteriological diagnosis of diphtheria: (1) cultures must be made from the throat and nose on *Loeffler's serum medium* (no other medium is suitable) and incubated at 37.5° C. for twelve to eighteen hours, and then (2) a smear must be made from the growth on the medium, stained with methylene blue, toluidine blue, or some special stain, and examined for the presence of organisms resembling diphtheria bacilli. If bacilli having the characteristic irregular form and staining, and parallel arrangement of *Corynebacterium diphtheriae* are present, the bacteriologist will report the culture as *positive.* If the culture was made from a patient having the clinical symptoms of diphtheria, it is not necessary to go further to establish the diagnosis, and it may be assumed that the organisms seen in the smear are in truth virulent diphtheria bacilli. Thus, in typical cases of diphtheria this presumptive diagnosis, which is sufficient for all practical purposes, can be arrived at within twelve hours after the culture is made.

Final diagnosis. Virulence tests. In order to establish the diagnosis beyond doubt, however, it is necessary to identify the diphtheria-like bacilli in the culture definitely, and to prove that they are virulent; *i.e.*, to show that they produce diphtheria toxin. In case the positive culture comes from a *healthy carrier*, it is essential that these further tests be carried out, because it would be unfair and useless to require the isolation of the individual if the organisms he is harboring are not virulent.

We have previously mentioned organisms called *diphtheroids.* Some of these bacilli resemble *Corynebacterium diphtheriae* so closely in shape and arrangement, that even the most expert bacteriologist cannot always distinguish them from true diphtheria bacilli by microscopic examination. In order to prove that the diphtheria-*like* organism in the mixed throat culture is actually a virulent diphtheria bacillus, *virulence tests* must be conducted on animals.

The simplest way to do this is to inoculate a small amount of a suspension of the organisms from the original Loeffler's medium throat culture *intradermally* into each of two guinea pigs, one of which has been protected by a previous inoculation of diphtheria antitoxin. If the culture contains virulent diphtheria bacilli, a characteristic inflammation will appear about the site of the intradermal inoculation in the unprotected animal, while no reaction will occur in the animal that received antitoxin.

Another way to perform a virulence test is first to isolate the suspected diphtheria bacillus in pure culture, then inoculate guinea pigs *subcutaneously* with this pure culture. If the organism is a virulent diphtheria bacillus, an unprotected animal will die in three to five days, while an animal protected by antitoxin will remain well.

Precautions in making nose and throat cultures. Nurses are often called upon to make the original cultures from the patient on which the whole procedure of bacteriological diagnosis depends. It is essential that these cultures be made properly. The following are the principal points to be borne in mind:

(1) Have at hand *small, sterile, cotton swabs,* and tubes of *fresh, sterile Loeffler's medium slants.* Do not use old, dried-up medium. Do not use blood agar.

(2) For nose cultures, pass a swab straight back into one nostril, remove, and, with the usual aseptic precautions, roll the swab gently over the *entire* surface of a slant. *Label culture carefully,* and *place in a 37.5° C. incubator at once.*

(3) For a throat culture get the patient into a good light, or use a flashlight to *illuminate the throat well, so that you can see exactly what you are doing.* Hold the tongue down if necessary with a tongue depressor or spoon handle. Be careful that the patient does not cough directly into your face.

(4) Pass a swab over *the inflamed part of the throat only,* and remove quickly *without touching it to the cheeks, tongue or lips.* Try to get a bit of the membrane if one is present; otherwise swab the reddened areas. It is essential to avoid picking up many bacteria from other places, because these organisms might overgrow and crowd out the diphtheria bacilli in the culture. In case the swab is thus contaminated, or in case you are not sure

that you reached the inflamed part, it is better to repeat the procedure with a new swab.

(5) Using aseptic precautions, roll the swab gently on a slant so as to *inoculate the entire surface of the medium.* It is better to use a separate slant for the throat and for the nose culture. *Label culture carefully*, and *see that the culture is placed in an incubator (37.5° C.) as soon as possible.*

In case no Loeffler's serum medium is available, the swabs themselves may be placed in a sterile tube and sent to the diagnostic laboratory.

Diphtheria antitoxin. *Preparation and standardization.* Diphtheria antitoxin is prepared commercially by inoculating healthy young horses repeatedly with increasing amounts of diphtheria *toxin* (not the germs). The injections are continued during several months, until the blood of the animals contains a large amount of antitoxin. The horses are then bled from the jugular vein and the blood is allowed to stand until a firm clot has formed. Then the clear, fluid *serum*, which has exuded from the clot, is collected. This crude horse serum contains the desired antitoxin, but before it is put on the market for use in human disease, the serum is greatly concentrated to remove most of the inert constituents, purified and carefully tested to be sure it is free of germs, and standardized so that its antitoxin content may be known.

Diphtheria antitoxin, like tetanus antitoxin, is standardized by testing its protective power for guinea pigs inoculated with the toxin. Manufacturers of antitoxin compare their product with a *standard antitoxin* kept in the government laboratories at Washington. First, a toxin is standardized by tests against this standard antitoxin; then, by use of this freshly tested toxin, the protective power of the unknown antitoxin for 250-gram guinea pigs is determined. The technical methods of arriving at a unit of antitoxin are rather complicated, but it will be sufficient to realize that *1 unit of diphtheria antitoxin will protect guinea pigs against considerably more than one hundred fatal doses of diphtheria toxin.* Commercial antitoxin usually contains about 1,000 to 1,500 units per cubic centimeter.

Prophylactic and therapeutic use. Diphtheria antitoxin is effective for both the prevention and the treatment of diphtheria. Susceptible persons who have been exposed to the infection may be given immediate protection by the subcutaneous injection of about 1,000 units of antitoxin, and thus avoid an attack of the disease. This, of course, is a passive immunization, and since the material injected is horse serum and really foreign to the human body, it is rapidly eliminated, so that the immunity lasts only for a short time. The prophylactic use of antitoxin in this way is now rarely necessary.

In the *treatment* of diphtheria, antitoxin is invaluable. When injected into the body, it combines with and neutralizes any diphtheria toxin it encounters in the blood stream, thus preventing the poisoning of vital tissues. It stops the progress of the disease and permits the patient to recover. Before the use of antitoxin, which began in 1895, as many as 30 to 50 per cent of cases resulted in death. Now the death rate in the general population averages about 5 to 8 in 100 cases. Most of the deaths now occur in persons who receive antitoxin *too late* or not at all, for it has been demonstrated repeatedly that *when antitoxin is given in sufficient amounts on the first day of illness, recovery is practically certain.* The great importance of early diagnosis is obvious. The amount of antitoxin which may be necessary to bring about a cure in any one case varies according to the age of the patient and the severity of the infection from 3,000 to 30,000 units or more.

Schick test for susceptibility to diphtheria. This test, first perfected by Schick in 1913, is really a test for the presence of diphtheria antitoxin in the blood. It is at the same time a test for susceptibility (or immunity) to diphtheria, since resistance to this disease depends principally upon antitoxin. The procedure consists in the inoculation of a minute amount of diluted diphtheria *toxin* intradermally into the skin of the forearm.

Toxin prepared commercially for the Schick test is supplied in diluted form, ready for use. The toxin solution contains *an amount of toxin in each Schick test dose* (0.1 c.c.) *equivalent to 1/50 of the M.L.D. for a guinea pig.* Of this toxin solution 0.1 c.c. is injected *intradermally* into the flexor surface of one forearm. For a *control,* a like amount of solution of *the same toxin, which has been heated to 75° C. for one hour,* is injected intradermally into the opposite arm. This heating destroys the toxin but does not change other substances in the solution which might cause an allergic reaction.

The changes occurring at the sites of inoculation are carefully watched for four or five days. The following types of reaction may be observed:

(*a*) *Negative Schick test.* No reaction in either arm. The injected toxin has been neutralized by the antitoxin in the blood and tissues.

(*b*) *Positive Schick test.* No reaction in the control arm. In the test arm a red flush appears after twenty-four to thirty-six hours, reaching its maximum development about the fourth day. At this time there is a circular circumscribed area, ½ cm. in diameter, slightly raised above the general surface of the skin. This inflammatory reaction is caused by the injurious effect of the toxin upon the skin. It fades slowly, finally leaving an area of brownish pigmentation.

(*c*) *Negative Schick test with pseudoreaction.* An inflammatory reaction, less sharply circumscribed than a true positive reaction, develops *equally on both arms within twenty-four hours.* It fades rapidly and is usually gone by the fourth day. This reaction is an *allergic* reaction and is due to sensitiveness to some constituent of the toxin solution.

(*d*) *Positive Schick test with pseudoreaction.* The control arm shows the changes described under (*c*)—the pseudoreaction. During the first twenty-four hours the test arm shows the same type of changes and the reaction is almost indistinguishable from that in the control arm. But after this time the reaction in the test arm continues to develop, while that in the control arm begins to fade. By the fourth day the difference between the two arms is usually quite definite.

It has been determined that a *negative* Schick test indicates the presence in the individual of *about 1/30 unit of antitoxin per cubic centimeter of his blood,* and experience has shown that this amount of antitoxin *insures immunity against diphtheria* under ordinary circumstances.

The results of Schick tests upon thousands of persons have largely explained why diphtheria occurs so often in children under the age of ten, but so rarely in adults. It has been found that 80 per cent or more of adults are immune. It has also been shown that the largest proportion of negative Schick tests—*i.e.*, the greatest percentage of immune individuals—is found among groups of persons who live in close contact with one another. Under these circumstances, the diphtheria bacilli are easily passed from person to person and there are likely to be many carriers. These *carriers immunize themselves, i.e.*, develop antitoxin in their blood, as the result of the absorption of small amounts of toxin from the germs in their throats. This is the bright side of the carrier problem. Most adults are resistant because they have carried about virulent diphtheria bacilli at some time or other long enough to have built up an immunity. It is easy to understand, therefore, why diphtheria is most frequent and most severe in young children.

Active immunization against diphtheria. To make individuals actively immune to diphtheria, it is necessary only to stimulate their tissues to form diphtheria antitoxin. But in order to do this the toxin must be safely introduced into the body. Diphtheria toxin is too dangerous to be used directly for the immunization of human beings without reducing its poisonous properties in some way. Toxin *mixed with antitoxin* was used as early as 1900 for the production of antitoxin in animals, but it was not until 1913 that Behring first reported the successful use of such *toxin-antitoxin mixtures* on human beings. Since that time the immunization of children with diphtheria toxin-antitoxin mixtures has been practiced very widely, especially in the United States, where the method was perfected largely through the work of Park and his collaborators in New York.

Several different methods of preparing the mixtures of toxin-antitoxin have been used, but in any case, the mixtures always contain a *slight excess of free, unneutralized toxin.* When the mixture is injected, this free toxin acts as the stimulus for the formation of antitoxin by the *individual's own body cells*—in other words, it starts the development of an *active immunity* against diphtheria. At the same time, the antitoxin in the injected mixture prevents any serious damage. Since this antitoxin in the original mixture is contained in horse (or goat) serum, it is gradually eliminated from the body. As this happens, more free toxin is slowly released, so that the stimulus afforded by a single injection extends over a considerable period of time. Three injections of 1 c.c. of the toxin-antitoxin at weekly intervals are usually given. There is almost no reaction, and thousands of children have been immunized by this method without the slightest harm. Toxin-antitoxin injections, however, successfully immunize only about 80 per cent of the vaccinated persons.

At the present time *diphtheria toxoid,* either plain or mixed with alum (the so-called *alum precipitated toxoid*) is preferred as the immunizing agent in place of toxin-antitoxin. It stimulates the development of a strong immunity in about 95 per cent of individuals receiving the injections. This material has the further advantage of containing no antitoxic serum and, therefore, it cannot sensitize the individual to horse serum. In young (pre-school) children (the persons most in need of immunization), neither the plain nor the alum-precipitated toxoid excites any appreciable reaction. Older children and adults, however, are often hypersensitive to proteins in the toxoids, and to avoid disagreeable local or general reactions, the toxoids may have to be diluted or the usual doses may have to be reduced. The alum-precipitated toxoid is more efficient than the plain toxoid, for when injected subcutaneously the material remains for several weeks in the injected area, and from this depot the toxoid is slowly absorbed, thus the stimulus to antitoxin formation continues for a long period. A single injection of alum-precipitated toxoid *usually* results in as high an antitoxin production as two or more injections of plain toxoid.

There is ample evidence that active immunization against diphtheria is highly successful in preventing the disease, although the vaccination may not protect every single individual. In communities where a considerable proportion of children have been immunized, the incidence of diphtheria among them has been very markedly reduced. All children should be given the vaccine treatment at about the age of 1 year. Active immunization against diphtheria is an especially valuable protection for nurses and doctors who must necessarily come in contact with many cases of the disease. It was not at all uncommon in former years for as many as 20 per cent of the nurses in infectious disease hospitals to develop diphtheria in the course of a year. This is now a rare occurrence in those hospitals where the routine immunization of all susceptible (Schick positive) members of the nursing staff is practiced.

It is important to remember that the immunity is not fully developed immediately after the injections have been given, but it becomes slowly stronger during succeeding weeks. The result of the immunization should be checked by the Schick test after about six months. The duration of the immunity varies in different individuals, but usually lasts for 4 to 7 years or longer.

Summary of the practical methods for the prevention and control of diphtheria. Diphtheria is typically a disease of schools and institutions where young children are herded together. The practical application of all measures for controlling diphtheria may be illustrated by the methods which would be used in the case of an epidemic in such an institution.

Any case of sore throat among the students is suspected as possibly diphtheria, and nose and throat cultures on Loeffler's medium are made at once. If the cultures show diphtheria-like bacilli and the student has had suspicious symptoms, a diagnosis of diphtheria is made. Then certain very definite procedures are carried out to care for the patient, and to protect his associates in the school and the community from the disease.

The patient is isolated, and he receives at once a sufficient dose of diphtheria antitoxin. The isolation is continued for about sixteen days or until 2 successive throat and nose cultures are negative for diphtheria bacilli.

Other students with whom the patient was in immediate contact during the week before his illness, are also segregated at once, and antitoxin is given them if they show any signs of coming down with the disease.

Nose and throat cultures are made from every individual in the school. If carriers are found, they are isolated until freed of the germs. Repeated cultures may have to be made and virulence tests performed upon the positive cultures from carriers to determine definitely the origin of the epidemic.

Schick tests are performed at once upon every individual. Those found susceptible are carefully watched and cultures are made again if any signs of infection develops. Those found to be Schick negative are immune, and, so long as they are not carriers, do not need to be isolated.

Active immunization of the susceptible individuals with toxoid should begin at once.

To avoid future epidemics the opportunities for the spread of germs should be reduced as much as possible by encouraging the development of personal cleanliness among the students. Carriers could be detected by cultures when they enter the insutution. Schick tests could be conducted routinely and susceptible persons could be actively immunized.

REVIEW QUESTIONS—CHAPTER XXX

1. Describe the clinical features of diphtheria and explain their cause. What is the origin of the term *diphtheria?*
2. Describe the morphological and staining characteristics of *Corynebacterium diphtheriae.*
3. What conditions are necessary for the growth of diphtheria bacilli in the laboratory? Name the medium most commonly used and give its composition. What peculiar advantages has this medium?
4. Discuss the resistance of diphtheria bacilli outside of the body.
5. Explain exactly what is meant by "diphtheria toxin." How is its toxic action tested?
6. What is meant by M.L.D.? What is the M.L.D. of diphtheria toxin for the guinea pig? Are all strains of diphtheria bacilli toxin-producers?
7. Define and explain the properties of *toxoid.* What is Ramon's *anatoxin?*
8. Explain how diphtheria spreads. Discuss the importance of healthy carriers of the bacilli.
9. Outline the necessary procedures for making a presumptive bacteriological diagnosis of diphtheria. Why is it important to have an early diagnosis?
10. Under what circumstances may a presumptive diagnosis be sufficient for practical purposes? When is a final diagnosis by virulence tests essential?
11. Outline the procedures necessary to make a final diagnosis of diphtheria and to prove the virulence of the organisms.
12. Outline concisely the necessary precautions in making nose and throat cultures. Explain why these precautions are essential.
13. Describe briefly (*a*) the preparation and (*b*) the standardization of commercial diphtheria antitoxin. Approximately what is the protective value of one unit of diphtheria antitoxin?
14. Describe the prophylactic use of diphtheria antitoxin.
15. Discuss the use and value of antitoxin in the treatment of diphtheria.
16. Explain precisely what is meant by a Schick test and how the test is carried out.
17. What types of reaction may occur in a Schick test? What is the meaning of each with respect to immunity and why?
18. What do the results of Schick tests show in regard to immunity among the general population?
19. What is the principle behind the methods of producing active immunity to diphtheria? What is *toxin-antitoxin* and how does it act to stimulate the development of immunity?
20. What other preparation is widely used for active immunization? What is alum-precipitated toxoid? Compare the advantages and disadvantages of toxin-antitoxin, plain toxoid, and alum-precipitated toxoid.
21. Discuss the practical value of active immunization against diphtheria.

What test should be performed, and when, to prove the immunization successful?

22. Outline the practical steps that should be taken in the event of an outbreak of diphtheria in a boarding school to (*a*) diagnose the case as diphtheria and treat the patient, (*b*) protect those who have been in immediate contact with the patient, (*c*) trace the source of the infection, and (*d*) determine the susceptible individuals in the school and protect them from possible infection. What could be done to avoid future outbreaks?

CHAPTER XXXI

INFECTION OF THE RESPIRATORY TRACT. COMMON COLDS. VIRUS DISEASES. WHOOPING COUGH.

BEFORE DISCUSSING infections of the respiratory tract it will be useful to call to mind the main features of the **anatomy** of these parts and its influence upon infection. The respiratory tract may be thought of as made up of three sections: (1) an upper part, including the *nose* and the accessory *sinuses* and the *nasopharynx*, (2) an intermediary part—the *pharynx*—which serves as a passage for food on its way to the oesophagus and stomach, as well as for air on its way to the lungs, and contains the *tonsils*, and (3) a lower part, including the *larynx*, *trachea*, *bronchi*, and *lungs*.

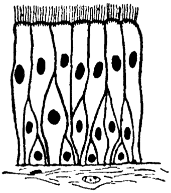

FIG. 70. Section through the wall of a bronchus, showing the ciliated epithelial cells. These cilia are in constant motion, carrying dirt and bacteria upward toward the mouth. The walls of the trachea and nasal sinuses are similarly lined with ciliated epithelium.

The nose opens externally through the nostrils and posteriorly into the nasopharynx (the upper part of the throat back of the soft palate). Each side of the nose consists of a rather large air chamber, completely separated from the opposite side by the nasal septum. The turbinate bones project into the nose from the sides, thus greatly increasing the total surface of the mucous membrane in the nose. From the top, the sides, and rear of the nasal cavity, openings lead to accessory air chambers, the sinuses. These sinuses are really the hollow interior of bones of the face. The nose and sinuses together make a series of communicating air chambers. The epithelial cells lining these chambers are ciliated, *i.e.*, equipped with tiny, hairlike processes called *cilia*. These cilia are in constant motion, sweeping dust and germs toward the nostrils (Fig. 70). From the nasopharynx there is a connection by way of the Eustachian tube with the middle ear.

By "tonsils" we usually mean the two almond-shaped bodies lying one on each side and at the back of the throat. These are not the only tonsils, however, though the largest. They are part of a ring of such structures which circles the throat at this point. They are really lymph glands. One of them is present normally in children on the roof of the nasopharynx. Sometimes this becomes very large so that it obstructs the free passage of air and makes breathing through the nose difficult. It then constitutes what is called *adenoids* (Fig. 71).

The great importance of natural nose-breathing in reducing the possibility of respiratory infections and in preserving the general health is strikingly shown by the long list of troubles which follow when adenoids, deviated nasal septum, or other pathological condition makes an individual a habitual mouth-breather. When the inspired air passes first through the nose it is made moist and warm, and almost entirely free of dust and germs. It seems that only by this route can the air reach the lungs in proper condition. Mouth-breathing often leads to chronic sore throat, and obstructions in the nasal passages greatly increases the tendency toward infection of the whole respiratory tract.

The larynx is a stiff, cartilaginous box, containing the delicate vocal cords. It joins the trachea below, a rigid tube which soon divides into two primary bronchi, one leading to the lung on each side of the chest. The bronchi divide and subdivide, like the branches of a tree, into smaller and smaller tubes, until finally each tiny bronchiole ends in a group of little, closed sacs, just about large enough to be seen with the naked eye (Fig. 73). The lungs, then, are really made up of myriads of these tiny air sacs, called *alveoli,* all joined together by a system of branched tubes which eventually unite with the trachea. The walls of the alveoli are very thin and contain numerous capillaries, so that the exchange of oxygen and carbon dioxide between the blood and the air in the alveoli—the essential function of the lungs—can go on readily. In the human body the right lung is divided into three *lobes,* and the left into two lobes.

The bronchi and the trachea are lined, like the nose and sinuses, with ciliated epithelium. These cilia are constantly acting to clear the whole bronchial tree of dirt and germs by sweeping mucous up to the mouth where it may be swallowed or expectorated. The action of these cilia probably is an important factor in protecting the lungs from infection.

Normal flora of the nasal passages, and lower respiratory tract. In the nasal passages there are fewer bacteria normally present than in the mouth and throat. Staphylococci, especially

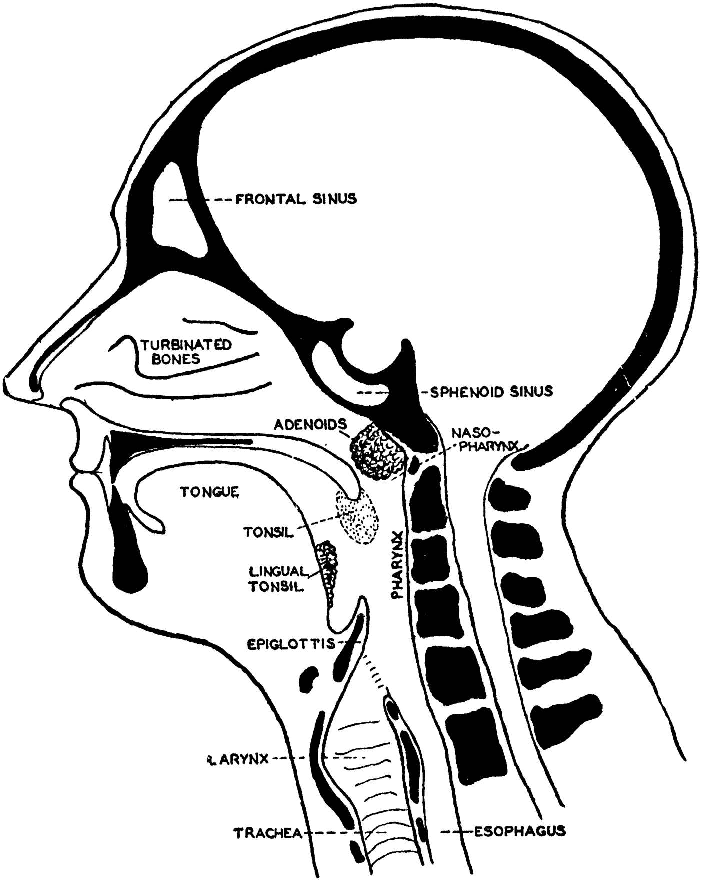

FIG. 71. Vertical section through the head and neck, showing the principal anatomical features of the upper respiratory tract.

Staphylococcus albus, and harmless Gram-negative diplococci (*Neisseria pharyngis*) are usually most abundant. Indifferent colony streptococci, *Streptococcus viridans*, and diphtheroids are usually present also. These organisms seem to constitute the normal bacterial population. About half the individuals in the general population of cities may be harboring in their noses potentially pathogenic organisms, such as the *Pneumococcus*, and *Hemophilus influenzae*, and a smaller proportion may be carrying *Streptococcus hemolyticus* or *Corynebacterium diphtheriae*. The *nasopharynx* is especially likely to harbor pathogenic organisms, if any are present, and the bacteriologist, when looking for carriers of *Meningococci*, *Streptococcus hemolyticus* or other pathogens, takes particular pains to culture this area.

The few studies that have been made indicate that the sinuses are normally sterile. The healthy trachea and bronchi contain very few, if any, bacteria.

Common colds. Recent studies among factory workers, students, and others have shown that by far the most frequent cause of illness, year in and year out, is inflammation of the upper respiratory tract. "Colds" in one form or another, cause more loss of time from work or study than any other ailment. Few individuals escape an occasional cold, and many have three or more every year. The common cold can fairly be regarded as a kind of modern plague.

Some colds are highly communicable, and spread very rapidly from person to person by contact, especially through droplet infection. Others seem to occur as a result of lowered resistance in certain individuals, and are less often passed on to other persons.

The most recent bacteriological studies indicate that common colds, of the communicable types at least, are *probably caused by a filtrable virus.*

It is probable that colds are most readily passed to other persons during the earliest stage of the infection, and before the nose becomes blocked by pus. In these early stages comparatively few bacteria are present. In the later stages of colds, however, some of the potentially pathogenic organisms so commonly found in the healthy respiratory tract are usually present in large numbers, and one or more of them may be responsible for spread of the inflammation from the nose to the sinuses, ear, throat, larynx, or deeper parts. The most prominent of these germs likely to complicate colds are the *Pneumococcus, Hemophilus influenzae, Streptococcus hemolyticus*, and *Staphylococcus aureus.*

The fact that these and other dangerous organisms are likely to be present during the later stages of a cold, is very significant and explains why colds, though seemingly trivial, are nevertheless a serious menace to both the individual and the public health. The inflammation of the nose, throat, and upper bronchi which accompanies a cold prepares these places for the lodgment and multiplication of highly virulent germs and the cold may lead to a far more serious disease, such as pneumonia. From the public health viewpoint, colds are important because germs of any kind which may be present in the respiratory passages of infected persons become distributed among all others with whom they come in contact, as a result of the inevitable sneezing, coughing, and spitting which accompanies the cold. In schools, army camps, and similar places, epidemics of colds are likely to be followed by outbreaks of measles, meningitis, diphtheria, or other diseases in which the germs are transmitted through nose and throat secretions.

The spread of colds can be prevented only if extraordinary care is taken *during the earliest stages of the infection*, as well as later, to avoid the transfer to others of secretions from the nose or throat. Well children should be kept away from children with colds. In schools, institutions, factories, army camps, and hospitals, the prompt isolation of all individuals who develop colds, so far as this may be possible, will greatly reduce the number of cases, and what is still more important, it will help to prevent the spread of more serious epidemic diseases.

A nurse who develops an acute respiratory infection must be relieved from duty at once as a measure of protection for herself as well as for her patients.

The avoidance of colds is a matter of real importance to the nurse. She should *try to avoid infection* by keeping away from persons with colds, and she should *try to avoid* excessive fatigue, exposure to cold and wet, and other *predisposing causes of colds.* An excellent rule, though perhaps hard to follow, is *to go to bed* *as soon as one feels a cold coming on.* This would prevent spreading of the infection and at the same time would help toward a rapid recovery.

Mixed vaccines, containing several of the common pathogenic organisms found in respiratory infections, have been used for the prevention and treatment of colds, but their value is uncertain.

Other forms of respiratory infection. *Sinusitis.* When infection extends from the nose into one of the sinuses and sets up an inflammation there, with pus-formation, we have the rather common disease called sinusitis. The condition is serious and has harmful effects in many ways upon the whole body. The inflammation often persists for months or years. The treatment is to open the sinus and so permit the pus to drain away. Any one of the several germs we have mentioned in connection with colds, or a mixture of them, may be responsible for sinus infection.

Tonsillitis. We have already mentioned that most cases of acute tonsillitis are caused by hemolytic streptococci. We have also noted that the germs of diphtheria, Vincent's angina, and syphilis frequently produce lesions on the tonsils. The tonsils are very often the seat of a chronic inflammation and a source of trouble for the whole body. They may act as a focus from which germs are carried to the joints, heart, or other organs. The removal of chronically enlarged tonsils is often followed by a marked improvement in general health as well as a reduction in the frequency of infections of the respiratory tract.

Laryngitis. Often infection spreads downward from the throat into the larynx. The walls of the larynx are rigid and cannot expand, so that when any considerable amount of pus and fluid collects there may be a serious interference with the breathing. We then have the condition called *croup.* The importance of laryngeal diphtheria has already been discussed. The larynx is sometimes the site of infection with the germs of syphilis or of tuberculosis.

Bronchitis. "Cold in the chest" may occur as part of general acute inflammation of the respiratory tract, and may be a complication of measles, influenza, or other disease. There is always the possibility that it may lead to pneumonia. The germs most likely to be responsible are those already mentioned as commonly present in colds. Chronic bronchitis is common in old persons.

Bronchiectasis. Sometimes, as a result of chronic bronchitis, the wall of a bronchus is weakened at one place, and it bulges out there to form a sac-like cavity (Fig. 72). In these cavities, masses of bacteria of both aerobic and anaerobic types, and of various species, accumulate and grow. Staphylococci, streptococci, other aerobes, and the anaerobic fusiform bacilli and spirochetes (from the mouth) are often numerous in the foul smelling sputum which collects in these cavities. When some of the sputum flows out into a normal bronchus, the patient coughs until the sac is emptied. His breath usually has a nauseating odor. Sometimes, when spirochetes are especially numerous in the sputum, the injection of salvarsan helps to reduce their number and makes the odor of the sputum less objectionable.

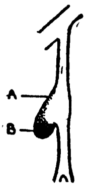

FIG. 72. Diagram to illustrate the condition called bronchiectasis. At A, the walls of the bronchus have become weakened and have dilated to form a sac-like cavity. B, mass of pus and bacteria which collects in the cavity.

Lung abscess. A localized inflammation in the lung tissue may occur as a complication of various disease conditions. Such abscesses, however, most commonly develop following an operation, and especially often after a tonsillectomy. These post-operative lung abscesses are apparently due to bacteria sucked into the lungs from the mouth while the patient is under the influence of the anesthetic. The pus which accumulates in them contains a great variety of organisms, but conspicuous among them in many cases are the anaerobic mouth bacteria, including *Bacterium melaninogenicum,* fusiform bacilli, and spirochetes. The infection often clears up spontaneously, but sometimes persists for months, and, unless effective surgical treatment can be given, it may eventually lead to a fatal bronchopneumonia.

Specific diseases of the respiratory tract. In addition to the disease conditions already mentioned there are several very important specific infections which primarily involve the respiratory tract. We have already discussed *scarlet fever, septic sore throat, diphtheria, Vincent's angina,* and *common colds.* Lung infection may be caused by *Actinomyces, Aspergillus, Blastomyces* or other fungi (Chapter XL). There remain to be considered *measles, mumps, psittacosis, influenza, whooping cough, pneumonia,* and *tuberculosis.* Of these the first four are *virus* diseases.

A considerable number of other communicable diseases are apparently transmitted from person to person by way of discharges from the nose and throat, though they are not primarily respiratory diseases. In this category belong *poliomyelitis* (infantile paralysis), *encephalitis*, and perhaps, also, chicken-pox and smallpox.

Measles. Measles is a highly communicable disease, characterized by catarrhal symptoms resembling an ordinary cold, fever, and the appearance of a characteristic skin rash. It is an exceedingly common disease all over the world. It spreads rapidly and seems to attack every susceptible person of whatever age who is exposed to it. Most cases occur in children, because the majority of adults have already had the disease and are immune. The incubation period averages about ten days. The infection is spread most readily toward the end of this period and *before* the characteristic rash appears.

The cause of this disease is probably a *filtrable virus*. It has been found that this virus is present in the blood at the height of the disease. It is also abundant in the mouth and nose secretions early in the disease, and with filtrates of these secretions measles has been reproduced in monkeys.

Uncomplicated measles is not a particularly severe disease, but, like influenza, it seems to reduce very markedly the normal resistance toward respiratory infection, so that secondary inflammation of the middle ear or other parts, due to staphylococci, streptococci, or other organisms, is liable to occur. The most dangerous complication is bronchopneumonia, and this is responsible for most of the deaths. It is important to remember that this serious bronchopneumonia may spread to other measles patients in a crowded hospital ward, unless isolation technique is carried out carefully.

Good results are obtained by the use of *convalescent serum* in the prevention and treatment of measles. If given early in the incubation period, it will entirely prevent the disease. If given later, it may greatly reduce the severity of the infection and help to prevent serious complications. Extracts from normal human placentas have been used in place of convalescent serum with apparent success.

Mumps. This disease is characterized by an inflammation and swelling of the salivary glands, especially the parotid glands on the side of the face. Epidemics of mumps spread with great rapidity and few susceptible persons escape. It is chiefly a disease of children in schools and institutions, but may occur in epidemic form among adults who are crowded together, as in army camps. It is usually not severe in children, but may be serious in adults, particularly in males who may have an extension of the inflammation to the testicles. Recovery usually leads to a lasting immunity.

Mumps has an incubation period of from two to three weeks. During this time while the patient is still up and about, the infection is readily passed to other persons; hence it is very hard to prevent the spread of the disease. The infection is transmitted by contact with mouth and nose secretions.

The cause of mumps is a *filtrable virus.* The disease has been reproduced in monkeys by injecting into the salivary glands of these animals a filtrate of the saliva from human patients.

Psittacosis. This is a *filtrable virus disease* of parrots that may be transmitted to man, and occasionally is spread from one person to another. The disease in human beings is serious and often fatal. There is fever, an acute lung involvement, and there may be paralysis. The virus is present in the nasal secretions and sputum. Psittacosis is highly contagious and there have been numerous infections among laboratory workers. Recovery confers a strong immunity.

The United States Public Health Service has set up rigid regulations to prevent the importation of sick parrots and also is exercising control over the breeding and sale of parrakeets in California, where the disease has apparently become endemic among these members of the parrot family.

Influenza. During 1914–19, influenza became pandemic and spread over the whole world. In a few months the disease destroyed more lives than were sacrificed during the four years of the War. It is still one of the most frequent causes of illness, and public health administrators are, at present, almost powerless to prevent its spread. The infection is evidently communicated by personal contact.

It is probable that influenza itself is a mild disease, producing only a slight inflammation in the respiratory tract, but causing a profound exhaustion, and as a result of this weakness, serious or fatal complications frequently follow. When death occurs, it is due to secondary infection; usually there is a bronchopneumonia.

The virus of influenza. In 1892 Pfeiffer isolated from the sputum of influenza patients the bacillus called *Hemophilus influenzae.* He regarded this "influenza bacillus" as the cause of the disease, and this was the general belief until very recently. In the past few years, however, it has been established that the primary cause of influenza is a *filtrable virus* and that influenza bacilli, and other bacteria, play only a secondary (though often a significant) rôle in bringing about the usual clinical disease. The *Hemophilus influenzae* is independently pathogenic, and a disease germ of considerable medical importance, as we shall see below, but not the specific infectious agent that initiates the disease we call influenza.

Although experimental results suggesting that influenza is a virus disease were reported previously by several other investigators it was the work of Smith, Andrewes, and Laidlaw in England (1933) with human influenza, and of Shope in the United States (1931) with swine influenza that laid the foundation for the fruitful studies of influenza virus now going on. The English workers succeeded in producing in ferrets an influenza-like disease by inoculating into their noses filtrates of the nasal or pharyngeal washings from human influenza patients. The disease was readily passed from ferret to ferret by intranasal inoculations or by natural contact. Serum from animals that recovered, and from human convalescents, neutralized the virus. These findings were soon confirmed by Francis at the Rockefeller Institute, and by others, and numerous strains of influenza virus have now been isolated in different parts of the world.

The entire study of the human influenza virus has been given added interest by the discoveries of Shope on swine influenza. This investigator showed that the primary cause of this disease is a filtrable virus, but that in the typical severe cases (as in human influenza) there is present in addition to the virus a bacillus closely resembling Pfeiffer's human influenza bacillus. The virus infection alone, however, was shown to confer a solid immunity to the natural disease.

By animal (protection) experiments it has been shown that the swine and human viruses are related, but not identical. The virus of the human epidemic influenza now prevalent is distinct from the swine virus. Many adults, however, who had influenza in 1918, have neutralizing antibodies in their blood for the swine virus, but not for the present human virus. Swine influenza first appeared in the United States at the beginning of the great pandemic of 1917–18, and it has been suggested that the influenza virus then prevalent in man was transmitted to swine, where it now persists, while the human virus now existing is a new, and different strain.

Hemophilus influenzae. The influenza bacillus is a small, somewhat pleomorphic, Gram-negative organism. It often appears as extremely short rods, but also may have the form of long slender filaments. It is nonmotile, and has no spores. It grows best aerobically, and at 37° C.

This organism is a so-called *hemophilic* (blood-loving) *bacillus,* because it will not grow in the absence of blood, unless certain essential growth-promoting factors present in blood are supplied from another source. It has been found that the growth of influenza bacilli depends upon two factors. One, called the X factor, is not destroyed by heating. It is found in red blood cells and is derived from the hemoglobin. The other factor, called the V factor, is destroyed by heat. It may be supplied by fresh extracts of red blood cells, vegetable tissue, or yeast. Certain animal and plant tissues, as well as blood, contain both necessary factors. Blood agar medium (especially the kind called chocolate blood agar) is used in the laboratory. The growth is delicate, and cultures die unless frequently transplanted.

The *Hemophilus influenzae* is often found in the healthy throat and nose, but it is potentially pathogenic, and is of considerable medical importance. Aside from its prominent part in the bronchopneumonia, and other complications, of *influenza,* it commonly appears as a secondary invader in still other disease conditions, such as the *pneumonia* following measles. It may cause *conjunctivitis, sinusitis, otitis media,* or *endocarditis.* The most important infection, however, for which the influenza bacillus alone may be responsible is a form of *meningitis* in children. Influenzal meningitis usually runs a rather protracted course, but is almost invariably fatal.

The so-called *Koch-Weeks bacillus,* the cause of the acute conjunctivitis known as pink-eye, though sometimes given another species name (*Hemophilus conjunctivitidis*) is probably identical with *H. influenzae.*

Immunity in influenza. It has generally been believed that immunity in man following an attack of influenza must be transient, because repeated attacks are likely to occur in successive years. It may be, however, that the resistance is actually more lasting, but is effective only against a particular strain of virus, and not against other strains that may appear in subsequent epidemics. At the present time several investigators, working with preparations of the virus, are attempting to develop an efficient vaccine for active immunization against influenza.

Whooping cough. This disease is an acute bronchitis, probably caused by a particular germ, first described by Bordet and Gengou in 1906 and called *Hemophilus pertussis.* After an incubation period of about two weeks, the disease begins like an ordinary cold, then in about a week the characteristic "whooping" cough begins. The whoop is caused by the effort of the child to recover his breath after a long paroxysm of coughing. The only result of this violent coughing may be the expectoration of a little mass of very sticky sputum. The disease often continues for a month or more. As in the case of other children's diseases, the greatest danger arises from complications, especially bronchopneumonia. Whooping cough is more dangerous than is generally realized. It causes more deaths than measles or scarlet fever, especially in infants. The disease spreads easily among children by personal contact.

Hemophilus pertussis. This organism is a small, somewhat oval-shaped aerobic, Gram-negative bacillus. It resembles the *Hemophilus influenzae,* but is a distinctly different germ. It is found in enormous numbers in the sticky sputum from cases of whooping cough. A special medium devised by Bordet and Gengou, consisting of a 1 per cent glycerine agar containing crushed potato mixed with an equal amount of rabbit or human blood, is used to secure the initial growth of the organism from the sputum. The bacillus grows slowly and feebly, and attempts to isolate it often fail. It is somewhat easier to obtain on so-called cough-plates. These are made by holding Petri plates of Bordet-Gengou medium 4 or 5 inches from the mouth of the patient while he coughs. The plates are then incubated in the usual way and examined for colonies of *Hemophilus pertussis* in about forty-eight hours.

Once isolated, the pertussis bacillus grows fairly readily on media enriched with blood or blood serum, and after a few generations may grow even on plain agar. In this respect it differs notably from *Hemophilus influenzae* which does not adjust itself to plain media, and requires the accessory growth factors X and V. The whooping cough bacillus differs from the influenza bacillus also in its failure to ferment carbohydrates, form indol, or reduce nitrates, as shown in the following table. The two species may also be distinguished by serological reactions.

	Dextrose Fermented	Maltose Fermented	Indol Produced	Nitrates Reduced	V and X Growth Factor Needed
H. influenzae	+	+ (most strains)	+ (most strains)	+	+
H. pertussis	–	–	–	–	–

The pertussis bacilli have no marked poisonous properties; they seem to exert their harmful effects by growing in masses on the walls of the larynx, trachea, and especially in the bronchi and bronchioles, causing the formation of a thick mucopurulent exudate.

Active immunization. A lasting immunity is acquired by those who recover from whooping cough. The seriousness of the disease in very young children has prompted various workers to attempt artificial active immunization.

Recently promising results have been obtained in the prevention of whooping cough with vaccines, such as that of Sauer, prepared from freshly isolated strains. For prophylaxis, infants should be vaccinated at about the sixth month, for the greatest mortality is during the first two years of life.

REVIEW QUESTIONS—CHAPTER XXXI

1. Review the anatomy of the respiratory tract and discuss its influence upon infection of these parts.
2. Name five kinds of bacteria that may be found normally in the nose or throat. Name four important pathogenic organisms that may be present.
3. Discuss common colds as to (*a*) their probable cause, (*b*) organisms likely to cause complicating infections, (*c*) frequency, and importance

from the point of view of both the individual and public health, (*d*) manner of spread, (*e*) prevention.

4. Name six other common forms of respiratory infection. Discuss the cause and nature of each.
5. Name three fungi that may cause lung infection. Name four virus infections and three bacterial diseases which primarily involve some part of the respiratory tract. Name other diseases which are apparently spread through nose and throat secretions.
6. State briefly the characteristic clinical features of measles. Discuss its (*a*) frequency and importance, (*b*) manner of spread, (*c*) cause, and (*d*) prevention.
7. State briefly the characteristic clinical features of mumps. Discuss its cause and manner of spread.
8. Describe briefly the chief features of psittacosis.
9. Discuss the clinical features and manner of spread of influenza. Who discovered *Hemophilus influenzae* and when? What are the prevailing ideas as to the relation of this organism to the causation of influenza?
10. Describe the discovery of influenza virus, and the principal properties of this virus. What is swine influenza? Explain the possible relation between human and swine influenza. Discuss immunity to influenza.
11. Describe the morphological and physiological properties of *Hemophilus influenzae*. Explain its peculiar food requirements. How may it be distinguished from *Hemophilus pertussis?* What is the Koch-Weeks bacillus?
12. In what disease conditions other than influenza, may *Hemophilus influenzae* be found? What fatal disease may it cause in children?
13. What are the characteristic features of whooping cough? Name and describe the causative organism.
14. How does *Hemophilus pertussis* differ from the influenza bacillus? What seems to explain its harmful effects?
15. Discuss active immunization against whooping cough.

CHAPTER XXXII

PNEUMONIA

BY "PNEUMONIA" is meant an inflammation of the walls of the alveoli, or air sacs, which make up the lung tissues, as a result of which the alveoli become filled with exudate. This disease may be *primary, i.e.*, a well person may suddenly develop an acute pneumonia. In this case, the infection almost invariably involves one *entire lobe*, or two or more entire lobes of the lung. This is called *acute lobar pneumonia.* Another form of pneumonia is *secondary, i.e.*, it occurs as a complication to some other disease condition. In this case the infection usually results from an extension of an acute bronchitis into the alveoli, and the lung is involved only in scattered patches about the inflamed bronchioles. This is called *bronchopneumonia.* Pneumonia is one of the most fatal of all germ diseases.

Secondary pneumonia. We have already pointed out that measles, whooping cough, and influenza are especially liable to be complicated by a secondary infection of the lungs in the form of a bronchopneumonia, and have mentioned that this complication is responsible for nearly all the deaths of patients with these diseases. This type of lung infection may also complicate a great many other diseases, and in fact, a terminal bronchopneumonia is the immediate cause of death in the great majority of cases of chronic tuberculosis, cancer, and various other pathological conditions. Though not always fatal, a secondary pneumonia is a very dangerous infection because the patient is usually greatly weakened by pre-existing disease.

No single kind of germ is responsible for all cases of secondary pneumonia and there is a mixture of bacteria in the inflamed lung, but in groups of cases occurring in the same place a particular species often predominates. Thus, *Streptococcus hemolyticus* is frequently the most abundant germ, while in other groups of cases *Pneumococci*, *Staphylococci*, or *Hemophilus influenzae* may be predominant. These germs are doubtless present in the throat before the disease begins, in many cases, and invade the lung only when the resistance of the patient becomes sufficiently low. Occasionally the bronchopneumonia is caused by the germ which is responsible also for the primary infection—as, for example, the typhoid bacillus in cases of typhoid fever.

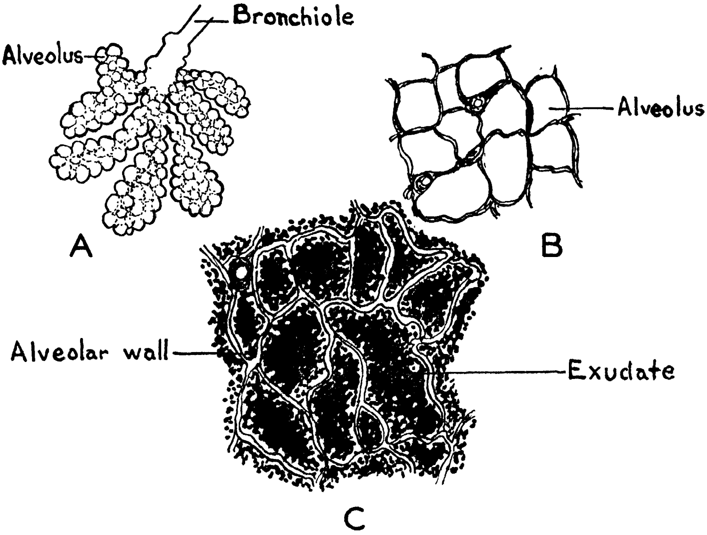

Fig. 73. A, diagrammatic representation of a terminal bronchiole and the group of tiny air sacs, or alveoli, attached. The lungs are made up of innumerable units of this nature. B, section of the normal lung, showing the thin-walled alveoli filled with air. C, section of the lung in lobar pneumonia, showing how the alveoli become solidly filled with a purulent exudate.

Acute lobar pneumonia. The onset of this disease is usually abrupt. A person who is feeling quite well suddenly develops a severe chill, pain in the side, then a high fever, and goes to bed at once. An acute inflammation involving whole lobes of the lungs has set in. The alveoli become filled with an exudate, consisting at first mostly of fluid and red blood cells, then later of leukocytes (Fig. 73). The affected lobe becomes much enlarged and is no longer a sponge of air cells, but a solid tissue, more like liver than lung. The patient is very ill, and death may occur in a few days. The majority of cases recover, however, and their recovery is usually associated with the phenomenon called the *crisis*, which occurs about the fourth to the tenth day. At the time of crisis, the temperature falls to normal and, within a few hours, there is a remarkable change for the better in the general condition of the patient. Convalescence then begins. The exudate in the alveoli rapidly disappears and the lung is soon able again to function normally. The cause of the crisis in pneumonia is not certain, but it is probably associated with the development by the patient of a sufficient amount of antibodies in his blood to counteract the harmful effects of the germ or its poison.

Occasionally, lobar pneumonia is caused by *Streptococcus hemolyticus*, *Staphylococcus aureus*, *Hemophilus influenzae*, or *Klebsiella pneumoniae* (a capsulated Gram-negative bacillus, generally known as *Friedlander's bacillus*), but by far the greatest number of cases of acute lobar pneumonia are due to a single kind of germ, the *Pneumococcus* (*Diplococcus pneumoniae*). This germ was discovered independently by Sternberg and by Pasteur in 1880, but it was not until the work of Fränkel and of Weichselbaum in 1886 that its relation to the causation of pneumonia was firmly established.

Diplococcus pneumoniae. *Morphology and staining.* The pneumococci are comparatively large, Gram-positive cocci, occurring in pairs, and shaped somewhat like the head of a lance or spear (Fig. 74). As seen in smears from the body tissues, each pair is surrounded by a wide capsule. The capsule is sometimes lost when the organisms are grown on artificial culture media. Occasionally, these bacteria form short chains and closely resemble the streptococci.

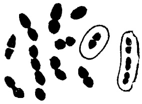

FIG. 74. The *Pneumococcus* (*Diplococcus pneumoniae*).

Physiological properties. The pneumococci, like the streptococci, are parasitic organisms, closely adapted to the living body, hence they require media enriched with blood or other animal fluids, and will grow readily only at body temperature. On blood agar, they form a colony surrounded by a greenish brown zone and difficult to distinguish from that of *Streptococcus viridans.*

Heat and chemical disinfectants destroy pneumococci readily. A noteworthy property of the organisms is their *solubility in bile.*

The clinical symptoms in pneumonia obviously suggest that the patient is suffering from a poisoning of some kind, but although many studies of this matter have been made, it is still not certain whether the pneumococci themselves produce toxic substances of any consequence.

Differentiation of pneumococci from streptococci. The similarity in the character of growth in culture media and in morphology sometimes makes it difficult to be certain whether a given organism is a *Streptococcus* or a *Pneumococcus.* The following are the principal differences between these organisms:

Pneumococci	*Streptococci*
Typically lance-shaped diplococci, with capsule.	Typically smaller cocci in chains, rarely capsulated.
Soluble in bile. All strains ferment inulin.	Insoluble in bile. Most strains do not ferment inulin.

Types of pneumococci and their importance in causing lobar pneumonia. In 1910, Neufeld and Handel, and later Cole, Dochez, and others, found that the various strains of pneumococci isolated from cases of pneumonia do not all stimulate the formation of exactly the same antibody. They showed that the serum of an animal immunized with a particular culture would not agglutinate all pneumococci, but only certain strains of these organisms, whereas the serum of an animal immunized with another culture would agglutinate other strains. They found that among the pneumococci there are three common types, *Type I*, *Type II*, and *Type III.* The serum of an animal immunized with a Type I strain of pneumococcus will agglutinate Type I organisms only. Similarly antiserums prepared with Type II and Type III pneumococci will agglutinate only pneumococci of the corresponding types. All the strains of pneumococci which do not fall into one of these three types were classed together in one group, *Group IV.*

Other workers have since subdivided *Group IV* by serological reactions, so that now no less than thirty distinct types of pneumococci are recognized. Several of these newly separated types have been found responsible for localized groups of cases of lobar pneumonia, among them Types IV, V, VII, VIII, and XIV, but the majority of the pneumococci that do not fall into one of the original three types are of relatively low virulence. They are the types found regularly in the *normal* throat, and are more likely to be the predominant organism in *bronchopneumonia*, than in lobar pneumonia. Types I, II, and III, on the other hand, are *not* common in the normal throat.

There is a considerable variation from time to time and from place to place in the frequency with which the various types of pneumococci are found in cases of lobar pneumonia. It is generally true, however, that Type I and Type II each account for about one third of all cases, Type III occurs in about 15 per cent of cases, and *Group IV* pneumococci (i.e., varieties belonging to one of the newly recognized, higher numbered types) are the causative organisms in about 25 per cent of cases.

It is generally agreed that the most severe cases of lobar pneumonia are those due to Type III pneumococci. This type has a conspicuously large capsule. From 33 to 50 per cent of the patients with Type III infection may die. The mortality in Type I and Type II infections is about equal, though some figures indicate that Type II may be on the average somewhat the more virulent. In different groups of cases from 20 to 40 per cent of patients infected with Types I or II may succumb. Usually pneumonias due to pneumococci of the higher numbered types, except in certain localized outbreaks in children, are distinctly less severe, and only about 10 to 15 per cent of cases are fatal. In any instance, a fatal issue seems to be closely correlated with the persistence of pneumococci in the blood stream. In at least a third of lobar pneumonia cases a bacteremia occurs; if this is transient eventual recovery is likely, but if persistent the patient is more liable to die.

The capsules of pneumococci. Only capsulated pneumococci are virulent and the specific character of the antibody produced by the different types of pneumococci is due to the different chemical structure of the *capsule* in each type. The capsular substance contains a complex carbohydrate, which differs with each type. This carbohydrate, or *specific soluble substance*, as it is called, acts as a partial antigen, or *hapten*, (p. 324), and in its combination with the protein of the body of the germ, is responsible for the specific character of the antibody produced by each type.

Bacteriological diagnosis of lobar pneumonia. The causative germs may be discovered (1) by *blood cultures*, or (2) by *direct smears and cultures from the sputum.*

Blood cultures. These are made in the usual way by taking 5 or 10 c.c. of blood from the patient's arm vein and introducing this directly into broth, or into agar for plates. All the organisms likely to be responsible for the infection will grow out readily, and the bacteriologist will have little difficulty in identifying them. Usually, of course, there will be a pure growth of the pneumococcus.

Sputum smears and cultures. Except in the occasional instances of pneumonia due to other organisms, the patient's sputum will contain conspicuous numbers of the causative pneumococci. The presence of capsulated pneumococci in sputum can often be established by microscopic examination of a direct smear stained by Gram's method or by some special capsule stain. Cultivation of the pneumococci directly from the specimen may be accomplished by first washing the tenacious sputum mass in several changes of sterile saline, then streaking the washed material over blood agar plates. Isolation of pneumococci from sputum is most easily done, however, by inoculating some of the washed sputum into the peritoneal cavity of a white mouse. This animal is so susceptible to pneumococci that it will die in a few hours, and in the peritoneal exudate, and the heart's blood, an almost pure growth of pneumococci will be found. Pure cultures of the organism on blood agar are then easily obtained. The germs may be identified as pneumococci by tests for inulin fermentation and solubility in bile.

Determination of pneumococcus types. It is not sufficient merely to determine that the patient is infected with pneumococci. *It is necessary to discover, also, what particular type of pneumococcus is present,* in order to know what therapeutic antiserum will be needed for treatment. Typing of the pneumococci is, therefore, always carried out, and the determination is made as quickly as possible.

Typing by mouse inoculation method; agglutination or precipitation tests. A widely used method for determining pneumococcus types is as follows. A small amount of the thick sputum coughed up by the patient is washed as free of the mouth bacteria as possible by rinsing it in sterile salt solution, then the washed sputum is inoculated into the peritoneal cavity of a white mouse. About eight hours later, the mouse, if not already dead, is chloroformed, an autopsy is performed, and smears and cultures are made from the peritoneal fluid and the heart's blood of the animal. Usually pneumococci will be found in enormous numbers and in practically pure culture. It is necessary only to wash out the peritoneal cavity with salt solution, to obtain a fairly heavy suspension of the germs. If now this suspension is briefly centrifuged to remove tissue cells and débris, then transferred to another centrifuge tube and centrifuged again at high speed, the clear supernatant fluid may be removed for precipitin tests, and the sediment of bacterial cells may be resuspended in salt solution for agglutination tests.

For the latter tests an amount of the pneumococcus suspension is mixed in a test tube with agglutinating serum specific for Type I, in another tube with an agglutinating serum for Type II, and in a third tube with an agglutinating serum for Type III. If agglutination of the pneumococci occurs in the tube containing Type I serum, the organisms must belong to Type I. If the agglutination occurs in the tubes containing Type II or Type III serum the germs must be Type II or Type III pneumococci respectively. If agglutination does not occur in any of the three tubes, the pneumococci belong to Group IV, i.e., to one of the higher numbered types.

To prove that the organisms in the suspension are actually pneumococci a separate tube may be set up to which *bile* is added; the organisms should dissolve, and the fluid in the tube should become clear.

Precipitin tests may be performed with this bile solution, or with the clear supernatant mentioned above. A few drops of the unknown pneumococcus solution are added to antiserums for each of the three principal pneumococcus types (and perhaps for other types as well) in such a way as to make a layer over the serum in each case. A precipitate at the junction of the fluids will occur only when the particular antiserum and the pneumococcus under test are of the same type.

Typing by the Neufeld "Quellung" method. Recently a faster and simpler method of determining the type of pneumococcus present in cases of pneumonia has become popular. This method depends upon the fact that when a capsulated pneumococcus comes in contact with its specific antiserum the capsule undergoes an almost immediate swelling, the so-called "Quellung" reaction. This reaction may be tested for as follows. A loopful of the patient's sputum is mixed in separate drops on slides with specific antiserums for the different pneumococcus types. A little methylene blue is also added to each serum-sputum mixture. The drops are covered with cover glasses or set up as hanging drops and examined with the oil immersion objective. When the serum and the pneumococcus are of the same type a marked swelling of the capsule appears within two to thirty minutes and usually there is little doubt of the result, since comparison of the other drops in which no capsular swelling has occurred will show a marked difference. This simple method has some limitations, but when properly applied it may be of great practical assistance in arriving at an early decision concerning the type of pneumococcus present. The manufacturers of biological products now furnish rabbit diagnostic antiserums for use in the Neufeld test for all of the pneumococcus types. The routine use of the method will eventually reveal the incidence and importance of the higher numbered types in the causation of labor pneumonia.

Such tests for the determination of type are reliable in direct proportion to the care taken in the collection of the sputum sample. Since harmless pneumococci are likely to be present in the normal mouth, an effort must be made to exclude these, and to get a sample of sputum which really comes from the inflamed lungs. The patient's mouth should be thoroughly rinsed, then he should be induced to raise sputum from the deeper bronchi by coughing. The sample should be collected in a sterile Petri dish or cup.

Serum treatment of pneumonia. The immune serums prepared against Type III pneumococci, although useful in identifying the type, do not have sufficient protective power against the germs to make them effective in treating pneumonia. Type I immune serum, however, is of proven value. Its use has reduced the death rate from Type I pneumococcus pneumonia from about 30 per cent to an average mortality rate of about 12 per cent. Type II serum has reduced the death rate in cases due to pneumococcus of that type from about 40 per cent to about 25 per cent. Encouraging results are reported in the use of antiserums against some of the higher numbered types. At the present time trials are being made with antiserums for these higher types prepared not by immunizing horses as usual, but by immunizing *rabbits*. There are a number of reasons why rabbit antiserum should prove more efficacious than horse antiserum, but as yet this matter is only in the experimental stage.

Antipneumococcus therapeutic serum is usually standardized by testing its protective power for mice inoculated with very virulent pneumococci. Some investigators have defined a *unit* of this serum as *ten times the smallest amount of serum, which will, on the average, protect a mouse against 100,000 minimum lethal doses of virulent pneumococci.* Mouse protection tests are not entirely satisfactory, however, and other methods for testing and standardizing antipneumococcus serums are now being tried in the hope of discovering a better procedure.

Treatment with sulfapyridine. This substance,—2(para-aminobenzenesulfamido) pyridine—a derivative of sulfanilamide, introduced in England in 1938, is apparently an effective remedy in pneumonias caused by any type of pneumococcus. It seems to inhibit the growth of pneumococci (regardless of type), and to be even more certain in its action toward pneumococci than sulfanilamide is toward streptococci. It will probably be used often in combination with a type specific antiserum.

Infections other than pneumonia caused by Diplococcus pneumoniae. Aside from their rôle in pneumonia, pneumococci are frequently the cause of serious infections of other kinds. During or following pneumonia, they may cause an *empyema* (inflammation with pus in the chest cavity), an *otitis media* or a *meningitis*, an *endocarditis* or *arthritis.* They also may be responsible for a *primary meningitis, or arthritis in children,* and they may cause a very severe *conjunctivitis* or other form of eye infection.

Prevention of pneumonia. It is probable that primary (lobar) pneumonia does not usually occur unless the individual is for some reason less resistant than normally. In other words, most human beings possess a high natural resistance to the common virulent types of pneumococci. Epidemics of lobar pneumonia are rare, except among crowded populations of poorly nourished people in mines, labor camps, and the like. Nevertheless, cases of lobar pneumonia in the hospital must be carefully isolated. Even though another case of lobar pneumonia is not likely to develop by contact with the patient, the pneumococci in the sputum, nose or ear discharges, etc., must be regarded as very dangerous germs, capable of causing serious infection. The nurse should take care not to breathe in particles of sputum sprayed into the air when the patient coughs. She must give especial attention to the disinfection of the sticky sputum and the numerous articles which will inevitably be contaminated with it.

Precautions against contact infection should be even more strict in the case of patients with bronchopneumonia. Dangers of carrying virulent streptococci or pneumococci from bed to bed in a ward of influenza or measles patients should be guarded against by the wearing of gauze masks, and the faithful practice of the rules of hand disinfection.

To reduce the mortality from lobar pneumonia it is imperative that the pneumococci be *typed promptly* in every case, and that the proper therapeutic antiserum be given to the patient at once. Many communities in the United States have established local *typing stations* where physicians may send specimens to be typed, and also have made available free supplies of therapeutic serum, including serum against the pneumococci of the higher numbered types.

Prophylactic immunization against pneumonia has been tried on a large scale among groups of young men in camps, mines, and hospitals, with good results. One of the latest forms of vaccine is made by chemical treatment of pneumococci and its essential constituent is not the intact organisms (as in the usual bacterial vaccine) but the soluble specific substance from the virulent types, in an antigenic form. The vaccinated individual is thus stimulated to develop the needed specific anticapsular antibodies. The relative immunity produced by pneumococcus vaccines probably lasts only a few months.

REVIEW QUESTIONS—CHAPTER XXXII

1. What two types of pneumonia are there from the anatomical point of view? What is meant by primary pneumonia, secondary pneumonia?
2. What is the importance of bronchopneumonia in connection with other diseases? What organisms may be responsible? What may be the origin of the infection?
3. Describe the principal features of acute lobar pneumonia.
4. Name five organisms that may cause lobar pneumonia. Who discovered the *Pneumococcus* and who proved its relation to the causation of pneumonia, and when?
5. Describe the morphology and staining, and principal physiological properties of pneumococci.

6. What species of the streptococci forms a colony on blood agar similar to that of pneumococci? What are the principal differences which serve to distinguish streptococci from pneumococci?
7. What is meant by the "type" of pneumococcus? How were these types originally discovered? How are the principal types designated? What is "Group IV"?
8. Where are the pneumococci of the different types to be found and approximately what proportion of cases of lobar pneumonia are caused by each type of pneumococcus? Which types are the more virulent?
9. Explain the part played by capsules of pneumococci in (*a*) determining the specificity of types, (*b*) determining virulence. What is the *specific soluble substance?*
10. Under what circumstances may pneumococci be found in cultures from the blood?
11. How may these organisms be isolated from the sputum of the patient? Why is it necessary to determine the type of pneumococcus causing the infection?
12. Outline briefly the principal steps in determining the type of pneumococcus (1) by the mouse inoculation method using agglutination on precipitin reactions and (2) by the Neufeld capsule-swelling method. Why is the latter method of special practical value? Why is it important to secure the sputum sample properly?
13. Discuss the value of serum treatment in pneumonia. What animals are used to produce antisera for the higher-numbered pneumococcus types? How is antipneumococcus serum standardized? What chemical is now being used for treatment?
14. Name eight infections, other than pneumonia, that may be caused by pneumococci.
15. Discuss the prevention of pneumonia. What measures are needed to reduce the mortality from lobar pneumonia?
16. What is the essential nature of the vaccine now being tried for active immunization against pneumonia?

CHAPTER XXXIII

EYE AND EAR INFECTIONS. MENINGITIS

INFECTIONS OF THE EYE

THE EYES are covered with a delicate transparent membrane called the *conjunctiva*. There are only a few bacteria which make up the usual flora of the normal conjunctiva. Conspicuous among them is a bacillus which looks very much like the diphtheria bacillus, though it is really a different species, and quite harmless. It is called *Corynebacterium xerosis*. *Staphylococcus albus* and a few other organisms may be present also. These organisms do no damage to the normal eye. But in the rare condition known as *xeropthalmia* (caused by lack of vitamin A in the diet), the secretions which normally lubricate the eyeball dry up, and these bacteria, and others of low virulence, may cause a severe inflammation.

The tears have a mild bactericidal power. This is too slight, however, to protect the eyes against infection with a really virulent germ.

Eye infections. The most important eye infections include *stye* and other inflammations of the eyelids, *ulcerations of the cornea* (the transparent outer wall of the eyeball), various forms of *conjunctivitis*, *gonorrheal ophthalmia*, and the disease called *trachoma*.

A *stye* is a staphylococcus infection of one of the sebacious follicles of the eyelashes. It is most likely to develop on the lower lid near the corner of the eye, and is associated with a chronic inflammation of the margin of the lids.

Ulcerations on the cornea may be due to various bacteria. Pneumococci are said to be the cause in many cases. Inherited syphilis in children may be responsible for a chronic inflammation in the cornea. A small Gram-negative bacillus, called the bacillus of Zur Nedden, has been described as the cause of corneal ulcers.

Conjunctivitis. Several varieties of bacteria may cause an acute, or subacute, conjunctivitis.

Acute epidemic conjunctivitis—so-called "pink-eye"—is caused by an organism named the *Koch-Weeks bacillus,* after the men who first described it. This germ is a small, Gram-negative, hemophilic organism, and is probably identical with *Hemophilus influenzae.* "Pink-eye" is contagious and may spread rapidly among members of a family or other group. In children the infection may accompany or follow measles or other common diseases.

Pneumococci may cause a serious, acute conjunctivitis. This disease is not common, although epidemics among children may occur. The infection is persistent, and very troublesome, but rarely leads to any permanent damage to the eye.

A subacute or chronic form of conjunctivitis, especially noticeable about the corners of the eyes (*angular conjunctivitis*) is caused by a short, thick Gram-negative organism called the *Morax-Axenfeld bacillus.*

There sometimes occurs a condition known as *follicular conjunctivitis,* characterized by the appearance of so-called follicles or granulations on the underside of the lids. Its cause is unknown. Granulations of the same nature occur in the earlier stages of trachoma, and it is suspected that there may be some relation between this simple follicular conjunctivitis and true trachoma.

Gonorrheal ophthalmia. Inflammation of the eyes by the germ of gonorrhea (*gonorrheal ophthalmia*), and particularly the form of this disease which occurs in newborn babies (*ophthalmia neonatorum*) is the most important of the acute eye infections. Children born of mothers with gonorrhea are almost certain to develop this infection, unless preventive measures are promptly taken. The gonococci (*Neisseria gonorrheae*) get into the eyes as the baby passes through the birth canal. The organisms cause severe injury to the eye tissues which often results in blindness. This form of eye infection is easily prevented, however. It is only necessary to instill a drop of a 2 per cent silver nitrate solution into the eyes immediately after birth (Credé method). This simple treatment is now given to *every* newborn baby whether the mother is suspected of having gonorrhea or not, and cases of gonorrheal ophthalmia in the newborn are now rare. At the same time the treatment prevents other possible eye infections.

Trachoma. This is a chronic disease of the eye which begins as a follicular conjunctivitis (granular lids), and leads eventually to formation of scars and other changes which result in partial or total blindness. The disease is common in many parts of the world, particularly in Egypt, China, and other Eastern countries. There are numerous cases also in the United States among the Indians and also among the native white people in certain sections. It is a communicable infection and spreads by contact from person to person in schools, asylums, and institutions, and among members of the same family. It is definitely associated with uncleanliness, however, and it appears that the most *ordinary personal cleanliness will protect against infection.* Hospital patients with trachoma should be isolated, nevertheless.

Numerous micro-organisms have been thought, at one time or another, to be responsible for trachoma. In 1927, Noguchi reported the isolation of a minute Gram-negative bacillus, which he called *Bacterium granulosis* from cases of trachoma in American Indians. With this germ he was able to produce in monkeys and chimpanzees an eye disease closely resembling human trachoma. Noguchi concluded that this germ is the specific cause of trachoma in men. This has not yet been confirmed by other workers, however, and at present the true cause of the disease remains uncertain. It may be a filtrable virus.

INFECTIONS OF THE EAR

Otitis media. Earache, like toothache, is a more important matter than is commonly thought. It is caused by an infection, usually confined to the middle ear (Fig. 75), and may have serious consequences. Inflammation of the middle ear is called *otitis media.* This infection may be acute or chronic. It occurs most frequently in children as a complication of scarlet fever, measles, common colds, or other infections of the nose and throat, but also may occur in adults. In the case of an acute otitis media, it may be necessary to incise the eardrum to permit the exudate to escape. Otherwise the infection may spread and cause a mastoiditis or a meningitis and perhaps a fatal septicemia. Frequent, severe ear infection, or chronic otitis media, may result in deafness.

Streptococcus hemolyticus or *Staphylococcus aureus* are the germs which most commonly cause otitis media. Bacteria of several othei species, such as the *Pneumococcus* or *Hemophilus* *influenzae*, may be found, however, and in many cases a mixture of three or four organisms is present.

The discharge from running ears may contain virulent germs, and must be carefully collected and destroyed.

MENINGITIS

By *meningitis* is meant an inflammation of the meninges, *i.e.*, the membranes which cover the spinal cord and brain. The strong outer membrane which lines the bony cavities of the skull and the spinal canal is called the *dura mater.* Just beneath this is another membrane, the *arachnoid.* The delicate inner membrane which immediately covers the brain and cord is called the *pia mater* (Fig. 76). Between the pia mater and the arachnoid is the subarachnoid space which contains the *cerebrospinal fluid.* In meningitis pus collects about the subarachnoid space.

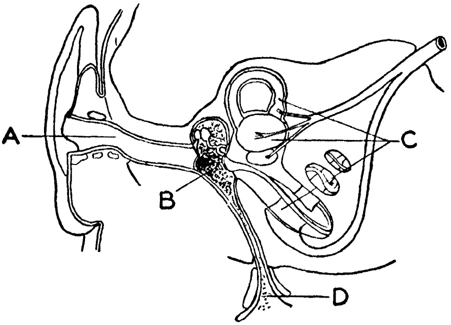

FIG. 75. Section through the ear, showing the condition in cases of acute otitis media. A, the external ear; C, the internal ear; D, the Eustachian tube; and B, the middle ear, shown filled with pus which causes the ear drum to bulge outward.

Meningitis occurs sometimes as a primary disease, and sometimes is secondary to disease in some other part of the body.

Secondary meningitis. The *Pneumococcus* not infrequently causes a meningitis, as a complication of lobar or bronchopneumonia, or otitis media, especially in children. *Streptococcus* meningitis is most frequently due to extension from a middle ear infection in children. A meningitis following a compound fracture of the skull is usually due to *Staphylococci* or *Streptococci.*

Many other organisms are found on occasions in different cases of secondary meningitis; indeed it seems that almost any bacterium, whether ordinarily pathogenic or not, may produce an infection when introduced by any means into the subarachnoid space. As a rule, all these forms of meningitis are rapidly fatal.

Primary meningitis. Illness characterized from the beginning by signs of meningitis may be caused by infection of the meninges with a variety of different bacteria and fungi. There are five kinds of bacteria, however, most commonly responsible for primary meningitis. These are the tubercle bacillus (*Mycobacterium tuberculosis*), the pneumococcus (*Diplococcus pneumoniae*), the influenza bacillus (*Hemophilus influenzae*), the hemolytic streptococcus (*Streptococcus hemolyticus*), and the meningococcus (*Neisseria meningitidis*). The latter is the cause of the serious and common communicable disease called *epidemic cerebrospinal meningitis.*

Tuberculous meningitis. This form of tuberculosis is less common now than in former years. It seems to occur in children as a primary infection, but probably in all cases there is a tuberculous focus in some other part of the body. In adults, tuberculous meningitis is seen more commonly as a terminal infection in cases of pulmonary tuberculosis. The disease is invariably fatal.

Laboratory diagnosis is made by examination of spinal fluid obtained by lumbar puncture. The fluid is usually fairly clear. The presence of tubercle bacilli can sometimes be demonstrated microscopically in smears of the sediment of the centrifugalized specimen, or in films prepared from the fine, web-like clot that forms characteristically when the fluid is allowed to stand for a few hours. Often there are too few of the organisms in the fluid to be found in this way. It is then necessary to inoculate some of the sediment from the centrifugalized sample into a guinea pig. After four to six weeks the animal is killed and the organs are examined for the presence of tubercles.

Pneumococcus meningitis. Nonepidemic primary meningitis is frequently caused by pneumococci. In these cases, the organisms are very abundant in the spinal fluid, which is cloudy and full of pus cells. The germs can be recognized in smears as Gram-positive diplococci, with capsules. It may be possible to type them by the Neufeld technique and prove their solubility in bile by direct tests upon the spinal fluid sample. They may be cultivated readily on blood agar, and their type may be checked by methods already described. Antipneumococcus serum is sometimes given, but the disease is usually fatal.

Influenzal meningitis. In children's hospitals this disease is generally encountered more frequently than any other form of nonepidemic meningitis. Diagnosis is usually made easily by direct smears from the cloudy spinal fluid. In these smears are seen very large numbers of faint-staining, Gram-negative rods, varying greatly in length from coccoid forms to long filaments. The marked pleomorphism is characteristic of the influenza bacillus in this situation. The organism may be isolated from the fluid on chocolate agar slants. The disease is commonly less acute than the other forms of meningitis mentioned here, but almost invariably the patient eventually succumbs.

Streptococcus meningitis. Almost as numerous in children as the cases of pneumococcus meningitis are those due to hemolytic streptococci. The Gram-positive cocci in pairs or chains are easily recognizable in direct smears of the spinal fluid, but their identification will require isolation on blood agar and serological and bile-solubility tests to exclude pneumococci. Formerly a hopelessly fatal infection, there is apparently a good chance to save many patients by treatment with sulfanilamide.

EPIDEMIC CEREBROSPINAL MENINGITIS

This is a specific, communicable disease always caused by the Gram-negative diplococcus called *Neisseria meningitidis* (the *meningococcus*), first studied by Weichselbaum in 1887. It frequently occurs in epidemic form in military camps and among groups of persons living in crowded quarters. Outbreaks of varying severity occasionally occur among the general population. The disease is fatal in a high proportion of cases, particularly in young persons.

The Meningococcus. *Morphology and staining.* Meningococci usually appear as small coffee-bean shaped Gram-negative diplococci. They may be capsulated. In cultures there is often great variation in size among the individual cocci and some stain more deeply than others. This is due to the fact that the organisms die and disintegrate very quickly, and many swollen and irregular-shaped cells are likely to be present in cultures only a few hours old. In the infected spinal fluid they are more regular in form and occur almost entirely within the cytoplasm of the pus cells, *i.e.*, they are *intracellular.* In shape and grouping and in their tendency to be intracellular in pus, they are practically indistinguishable from the *Gonococci.*

Physiological properties. Meningococci are strictly parasitic organisms and are cultivated in the laboratory with considerable difficulty. They require a medium enriched with blood or other body fluid. They will grow only at body temperature, and cultures must be maintained at 37° C. and transferred to new media frequently. The organisms are aerobic, but grow best when the oxygen tension is somewhat reduced.

As would be expected, they are sensitive to drying, moderate heat, and other injurious influences; they die in a short time at room temperature, and are readily killed by weak disinfectants.

The meningococci probably do not produce an exotoxin, but the bodies of the cocci themselves contain a strong *endotoxin*, which is released when the cells disintegrate. It is thought that toxic products of the organisms are responsible for much of the injury to the tissues that occurs in meningitis cases.

Mode of infection and spread of epidemic meningitis. It might well be asked how an infection localized in the subarachnoid space could become epidemic and spread by contact as meningitis does. The answer is that the meningococci are present not only in the infected spinal fluid, but also in the *nasopharynx* of cases and of carriers. The germs are passed from person to person chiefly by droplet infection. It is easy to understand, therefore, why *overcrowding* plays so important a part in the spread of the disease. It has been found that merely by increasing the space between beds in an army barracks during an epidemic, the number of carriers and of cases may be greatly reduced.

A striking feature of meningitis epidemics is the small number of cases of the disease (though these show a high mortality) in proportion to the number of individuals who are exposed to the germs. Different persons show a great deal of variation in susceptibility, but in general, resistance to the infection seems to be rather high. There is also, of course, a variation in the virulence of different strains of meningococci, and many strains do not seem able to invade the body easily. It follows as a result of these conditions, that in any group of persons exposed to contact with the germs, a large number will become *carriers* of the organisms, rather than victims of meningitis. There may be ten times as many carriers as cases of the disease. These carriers are the principal sources of infection for susceptible persons. The organisms often remain in the nasopharynx of carriers for a month or more. In nonepidemic times it is estimated that about 5 per cent of healthy persons may be meningococcus carriers.

Once having reached the nasopharynx of a susceptible person, it is not certain just how the organisms travel to the meninges. Most likely the cocci get into the blood stream and are thence deposited upon the meninges.

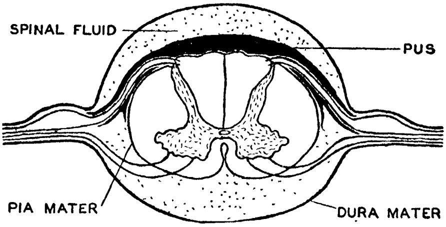

FIG. 76. A section through the spinal cord from a case of meningitis. The pus is found about the pia mater in the subarachnoid space. The spinal fluid is increased in amount and cloudy because of the presence of pus cells and bacteria. (Adapted from Emerson's *Essentials of Medicine*. Courtesy of J. B. Lippincott Company.)

The incubation period is about seven days, sometimes longer. As the germs develop on the meninges, pus collects, especially about the base of the brain, and the spinal fluid is increased in amount, causing abnormal pressure (Fig. 76). This accounts for some of the symptoms. The meningococci are present in the blood stream in the early stages of the disease and may cause secondary lesions in internal organs.

Sometimes in children there occurs an overwhelming blood invasion by meningococci, which progresses so rapidly that the child dies in only a few hours' time.

Laboratory diagnosis and detection of carriers. The laboratory diagnosis of epidemic meningitis depends upon the finding of meningococci in the spinal fluid. The germs may also be cultivated from the nasopharynx and sometimes from the blood.

Spinal fluid is collected by inserting a sterile lumbar puncture needle into the spinal canal; the fluid is usually turbid and contains many pus cells. A smear made from the sediment after centrifuging the fluid will show the typical Gram-negative diplococci, most of them within the cytoplasm of the leukocytes. It often happens, however, that there are no recognizable organisms present in the fluid, because the meningococci have gone into solution. In view of this fact, if Gram-positive cocci, influenza bacilli, or other organisms are *not* found, it is safe to assume that the infection is due to meningococci. Cultures of the germs are obtained by streaking the fluid over plates or slants of fresh blood agar or chocolate agar previously warmed to body temperature.

Blood cultures are made by drawing some of the patient's blood from an arm vein and adding it directly to a flask of warmed, infusion glucose broth, or brain medium.

Cultures from the nasopharynx are obtained by use of a special type of swab, made by the so-called West tube method (Fig. 50). These swabs are designed especially for obtaining material from high up in the nasopharynx, behind the soft palate, without contamination with bacteria from the lower pharynx or mouth. After this swab has been rubbed over the nasopharynx, it is streaked at once upon a warmed blood agar plate. This method of culturing from the nasopharynx is the routine method used for the *detection of carriers* of meningococcus.

The identification of the meningococci obtained in cultures and their differentiation from the several varieties of harmless Gram-negative diplococci found normally in the throat depends upon recognition of their somewhat characteristic colonies on blood agar, their failure to develop on simple culture medium or at room temperature, their capacity to ferment glucose and maltose, and their agglutination by antimeningococcus serum.

Serum treatment. The serum treatment of meningitis is recognized as a valuable measure for reducing mortality from this disease. The serum is prepared by immunizing horses. The animals are given a series of injections of a mixture of meningococci of several different strains, representing the principal types of the organism. There is no laboratory method by which the therapeutic value of this antimeningococcus serum can be tested accurately, but when the serum will agglutinate meningococci in a certain dilution, it is considered ready for use.

The serum is introduced directly into the spinal canal of the patient at the time lumbar puncture is made, somewhat less serum being injected than the amount of spinal fluid withdrawn. The serum may also be injected intravenously. When the treatment is given early in the disease, the results are favorable. The general use of this serum has reduced the number of deaths from meningitis from about 70 per cent to about 30 per cent of cases.

Sulfanilamide has been shown to be an effective therapeutic agent in meningococcus meningitis, and a combination of this drug with antimeningococcus serum will probably prove to be the most efficient treatment yet discovered.

Prevention. Early recognition of the disease and prompt isolation of the patient will go a long way toward preventing the spread of the infection. The isolation should continue for at least fourteen days, or until the germs have disappeared from the nasopharynx. The nurse and doctor must take precautions to avoid breathing in droplets expelled from the patient's nose and mouth. Concurrent disinfection of discharges from the nose and mouth, and articles soiled by them must be carried out.

In boarding schools, institutions, military barracks and similar places, the essential step in preventing epidemics of meningitis is to reduce overcrowding as much as possible, particularly in the sleeping quarters. General body resistance should be maintained as high as possible. In case of an outbreak in an institution, carriers should be found and isolated as long as they harbor the germs.

REVIEW QUESTIONS—CHAPTER XXXIII

1. What organisms are normally found on the conjunctiva? What may happen when the secretions which normally bathe the eyeball dry up? Name a condition in which this occurs.
2. Name five important forms of eye infection.
3. Describe the nature and the cause of (*a*) styes, (*b*) ulcerations of the cornea.
4. What is "pink-eye"? How does this infection spread?
5. Name another organism causing acute conjunctivitis.
6. Describe a common form of subacute or chronic conjunctivitis and name the causative organism.
7. What is follicular conjunctivitis?

8. Describe gonorrheal ophthalmia in the newborn. How is this infection prevented?
9. Describe the occurrence, manner of spread, and possible cause of trachoma.
10. What is *otitis media?* When may it occur? What organisms may cause it?
11. What is meant by *meningitis?* What are the meninges?
12. What organisms may cause a secondary meningitis and under what circumstances is this infection likely to occur?
13. Name the five most important organisms which may cause a primary meningitis. What is the cause of epidemic cerebrospinal meningitis?
14. Describe tuberculous meningitis. How is a bacteriological diagnosis made?
15. How could a bacteriological diagnosis of pneumococcus meningitis be made?
16. What is the importance of influenzal meningitis? Streptococcus meningitis? How is the diagnosis made in each case?
17. Who first studied the *Meningococcus* and when?
18. Describe the morphology and staining of meningococci, and their appearance in smears of infected spinal fluid.
19. Discuss the principal physiological properties of meningococci.
20. Describe the mode of infection and the manner of spread of epidemic meningitis. Explain the importance of carriers of the organism.
21. Outline the method of making a bacteriological diagnosis of epidemic meningitis. What method is used to detect meningococcus carriers? Describe the West tube.
22. What tests are used to identify an organism as a *Meningococcus?*
23. Discuss the use and value of antimeningococcus serum in the treatment of meningitis. How is this immune serum (*a*) produced, (*b*) standardized?
24. What chemical agent may be used to reinforce the serum?
25. Discuss the prevention of epidemic meningitis.

CHAPTER XXXIV

INFECTIONS OF THE GENITOURINARY TRACT. GONORRHEA. SYPHILIS

A CONSIDERABLE number of micro-organisms, of both aerobic and anaerobic types, are to be found normally upon the external genitals in both sexes. In the female an especially varied flora is always present, including aerobic diphtheroids, staphylococci, sarcinae, yeasts, colon bacilli and related organisms, and spirochetes, fusiform bacilli, and other anaerobes like those in the mouth. The harmless acid-fast bacillus known as *Mycobacterium smegmatis* is usually found in the genital region.

The normal flora of the vagina is a very limited one, probably because of the acidity of the normal vaginal secretions. A large aerobic Gram-positive bacillus, called *Döderlein's bacillus*, (probably identical with *Lactobacillus acidophilus*) is often the most conspicuous organism seen in vaginal cultures. It is not always present, however. There may be a few other organisms, such as diphtheroids, and Gram-positive cocci. Many of these are *anaerobic.*

The bacteria on the external genitals are ordinarily harmless. Some of them, however—the fusiform bacilli and spirochetes for example—are potentially pathogenic and may be responsible for local ulcerations or other inflammations. Infection of the uterus after childbirth (puerperal fever) is undoubtedly caused in some instances by bacteria normally in the vulva and vagina.

The internal genitourinary organs—bladder, ureters, kidneys, testes, uterus, and ovaries—are normally sterile.

Infections of the urinary tract. Acute or chronic infections, extending up the urethra to the bladder (*cystitis*) or involving the ureter and the pelvis of the kidney (*pyelitis*), are common. Young female children are especially liable to develop a urinary tract infection, probably because of the ease with which fecal material may be introduced into the urethra. The infection may persist in chronic form for many years. In adults, kidney stones, enlarged prostate glands, and other conditions which interfere mechanically with the urinary passages, often lead to a cystitis or pyelitis.

The organisms most frequently associated with these conditions are *Escherichia coli* and *Proteus vulgaris* and related intestinal bacteria. *Staphylococci* are also commonly found. Cystitis sometimes occurs as a complication of gonorrhea and is then due to the *Gonococcus*, alone or in association with other organisms. Tuberculosis of the kidney or bladder is often complicated by a secondary infection with staphylococci or colon bacilli.

To make a bacteriological diagnosis of cystitis or pyelitis, it is necessary to obtain a sample of urine with a sterile catheter. The sample is collected in a sterile centrifuge tube and, after it has been centrifugalized, the sediment is inoculated upon culture media and also examined microscopically. Urine obtained by catheter from a normal individual is clear and sterile, but in cases of acute infection of the urinary tract, it will usually contain many pus cells and should yield a pure culture of the causative organisms.

Puerperal fever. This disease, sometimes called childbed fever, is due to infection of the uterus after childbirth. The germs usually spread into the blood stream from the infected uterus, and a fatal septicemia often results.

The great majority of severe and fatal cases of puerperal fever are caused by *hemolytic streptococci* (rarely other organisms) *introduced into the uterus from without.* This type of puerperal infection was common before the introduction of aseptic methods into obstetrics. It was then the doctor, nurse, or midwife who innocently carried the deadly germs from patient to patient.

Oliver Wendell Holmes (1809–1894) was among the first to call attention to the infectious nature of puerperal fever in his celebrated essay "On the Contagiousness of Puerperal Fever" (1843). About the same time, a young Hungarian physician named Semmelweis (1818–1865) began to teach the same idea. He observed how frequently puerperal fever occurred in patients attended by students who were accustomed to go directly from the autopsy room to the maternity ward, and he came to the conclusion that uterine infection is caused by matter introduced into the birth canal from outside sources through the hands of the nurse, doctor, or other attendant. He succeeded in greatly reducing the amount of puerperal fever in his maternity clinics by the simple measure of requiring all attendants to wash and disinfect their hands before examining a patient. Other doctors were strongly opposed, however, to the ideas of Semmelweis, and his methods were never widely adopted nor well known until long after the independent work of Lister (beginning 1864) had finally brought about the general use of antiseptic technique. Semmelweis struggled with such fanatical zeal to get his methods accepted, that he finally lost his reason, and in 1865 was committed to an asylum. Curiously enough, at the time of this retirement, he was suffering from an infection of a wound on his hand, and in a short time he died of septicemia,—the very disease for the relief of which he had already sacrificed so much.

Nowadays, infection of the uterus after childbirth with bacteria introduced *from without* should never occur, and when it does, it is evidence of carelessness, or ignorance of modern aseptic technique, on the part of the midwife or other attendant. *Streptococcus hemolyticus* is usually the causative germ. Fatal streptococcus infections following criminal attempts to produce abortion are common. Other cases of postabortal infection are due to *Clostridium welchii,* the gas gangrene bacillus.

Puerperal fever is apparently caused in some instances not by germs introduced from outside sources, but by *organisms naturally present in the birth canal.* These bacteria are especially liable to be carried into the uterus in cases with long and difficult labor. They set up usually a mild infection, but sometimes cause a serious or fatal illness. It is this type of puerperal infection that obstetricians must deal with most frequently at the present time. In these cases, cultures from the infected uterus and blood often yield the same type of bacteria that are normally found on the surfaces of the vulva and vagina. Certain *anaerobic streptococci* are the predominant germs in many cases. *Bacterium melaninogenicum* is often present, accompanied by other anaerobes, in both the uterine and the blood cultures. The danger of uterine infection arising from these organisms normally present emphasizes the great importance of thorough disinfection of the external genitals before delivery.

Specific infections of the genital organs. Prevalence and importance of venereal diseases. There are three common infectious diseases which are usually localized, in their early stages, at least, on the genital organs. These are *chancroid*, *gonorrhea*, and *syphilis*. These diseases are generally referred to as *venereal diseases*, *i.e.*, diseases acquired by sexual contact, although by no means all cases arise in this way.

It would be difficult to overemphasize the importance of gonorrhea and syphilis. Together they constitute the greatest of perils, not merely to the individual sufferer, but to the happiness of the family and the future of the race. There are no wholly reliable figures as to the prevalence of these diseases, because only a fraction of all the cases are ever discovered by public health authorities, but there is no question that these infections are among the most widespread of human diseases. There are thought to be no less than six and one-half million syphilitics in the United States, and a much larger number of persons who either have or have had gonorrhea.

Although the germs of these diseases infect as readily the high as the low, it is true that cases are much more common among the lowest classes of the population. Various investigators have found that, from 35 to 40 per cent of criminal groups, and from 50 to 100 per cent of prostitutes are infected with syphilis. There are more cases of the venereal diseases among men than among women. Both gonorrhea and syphilis are more common in negroes than in whites.

Three other, less prevalent, specific infections that primarily involve the genital region are (4) *granuloma inguinale*, (5) *fuso-spirochetal infection*, and (6) *lymphogranuloma inguinale.*

CHANCROID

Chancroid is a common infection of the external genitals, characterized by multiple, small, painful ulcerations. The lesion is called a *soft chancre.* It somewhat resembles the primary sore of syphilis, but never becomes hardened as in the case of the syphilitic chancre. Often the inguinal glands become involved, eventually forming pus-filled buboes. Chancroid spreads almost entirely by sexual contact. It has no serious consequences and usually yields promptly to local treatment.

Hemophilus ducreyii. Chancroid is due to infection with a small Gram-negative, nonspore-bearing, nonmotile bacillus, first described by Ducrey (1889), and generally known as the *Ducrey bacillus.* The organism is now formally named *Hemophilus ducreyii.* In smears from the ulcerating sore, the bacilli appear as short, oval rods, in pairs or short chains. They are delicate, parasitic organisms, not resistant outside the body, but easily killed by dilute disinfectants or by unfavorable temperatures. As would be expected, they are difficult to cultivate in the laboratory, but they can be isolated on freshly made media enriched with blood.

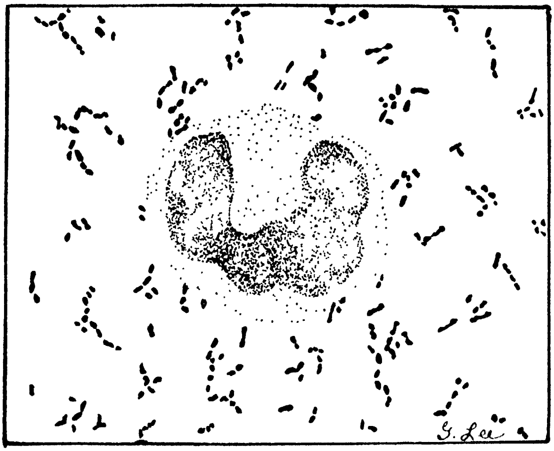

FIG. 77. *Hemophilus ducreyii* in the pus aspirated from a typical bubo in a patient suffering from chancroid infection. Drawing represents a single microscopic field.

Bacteriological diagnosis. Direct smears, cultures, and skin tests are used in the diagnosis of chancroid. Gram-stained *smears* made directly from the soft chancre, or from the pus aspirated from a bubo, reveal the typical organisms (Fig. 77). *Cultures* from the same materials may be made by inoculating freshly made blood media. *Skin tests* are made by introducing

0.1 c.c. of a suspension in saline of heat-killed *H. ducreyii* intradermally. If the patient has chancroid infection, and has become sensitized to the organism, an inflammatory reaction appears at the site of injection in about forty-eight hours.

GONORRHEA

Gonorrhea is a specific disease always caused by a particular species of Gram-negative diplococci, called *Neisseria gonorrheae* (the *gonococcus*). This organism was discovered by Neisser in 1879. It was first cultivated by Bumm (1885), and this investigator produced a typical gonorrhea in human beings by inoculating the urethra with pure cultures of this organism. Gonorrhea does not occur in any of the lower animals; it is strictly a human disease.

Neisseria gonorrheae. *Morphology and staining.* The gonococci are small, kidney-bean-shaped cocci, occurring in pairs, with the concave borders facing each other. The pair is commonly said to have a biscuit shape. They are practically indistinguishable in appearance from meningococci. Capsules have not been demonstrated. In pus from acute cases of gonorrhea, the gonococci are chiefly intracellular, *i.e.*, they occur *within the cytoplasm of the pus cells;* often 30 or more pairs may be crowded into a single cell (Fig. 78). They may also be found in small numbers outside the cells. They color intensely with the usual stains, and are frankly Gram-negative.

Physiological properties. The gonococcus, like the meningococcus, is strictly parasitic, and is even more difficult to cultivate in the laboratory. Various special types of media, enriched with blood or other body fluids, have been used for isolation of the cocci from pus, but none are universally successful. When a growth is obtained, it dies off quickly unless transplanted frequently to new media. Growth occurs only at body temperature and in the presence of abundant moisture.

An organism can be recognized as a *Gonococcus* by its form and staining, its failure to grow on the simpler culture media or at room temperature, and its capacity to ferment glucose, but not maltose. The failure to ferment maltose differentiates it from the meningococcus. Of course, the origin of the culture is an indication of the probable identity of the organism.

Gonococci probably do not produce any exotoxin. The disintegrated bodies of the germ are slightly poisonous, however, and this is evidence that they contain a mild endotoxin.

The difficulty in growing gonococci in the laboratory, and their failure to infect animals, makes clear how very closely these organisms are adapted to growth on the *living human body*. They will survive only for brief periods outside of the body. This is the reason why gonorrhea is almost always transmitted by direct bodily contact, and principally by sexual intercourse. Moderately high temperatures and weak disinfectants kill gonococci readily. It must be remembered, however, that *the germs may remain alive in pus on clothing or other objects for several hours,* or until the pus is completely dried.

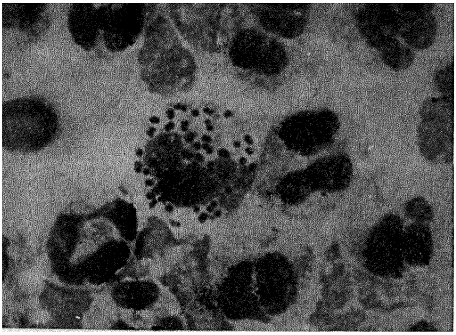

FIG. 78. *Neisseria gonorrheae* (Gonococci) in a direct smear of the pus from a case of acute gonorrhea, showing their characteristic occurrence within the cytoplasm of a pus cell. The large dark bodies in the photograph are the nuclei of the pus cells (polymorphonuclear leukocytes).

Clinical forms of gonorrhea. In adults, gonorrhea begins as an acute inflammation of the urethra, accompanied by a discharge of pus containing gonococci in great numbers. The inflammation usually appears within a week after the infection has been acquired. If the disease could always be successfully treated at this stage, it might not be greatly feared. But the inflammation almost invariably spreads, extending into the deeper structures, including the organs of reproduction. It becomes *chronic,* instead of acute, and chronic gonorrhea is an obstinate, treacherous disease, very resistant to treatment. In the chronic stage there may be little or no discharge. The germs are not so numerous, but they are producing deep-seated degenerative changes in vital internal organs.

The effects of chronic gonorrhea are very serious, both for the individual and for society. Gonorrhea is the prime cause of sterility—the condition in which conception cannot occur—resulting in childless marriages. This condition is brought about by injury to the reproductive organs themselves, or by blocking of the passages through which the reproductive cells—the male sperm and the female ovum—must travel. In women, chronic gonorrhea is responsible for a long list of troubles, some requiring surgical treatment, and a large proportion of the patients who come to the gynecological clinic are suffering from the effects of this disease. The gonococci may invade the blood stream, and give rise to many complications, such as a severe arthritis or endocarditis. The gonococci are, in fact, among the most virulent germs known.

The complete cure of gonorrhea, especially if effective treatment is delayed until the disease has become chronic, is difficult and uncertain. Neither serums nor vaccines can be used to any advantage. Many persons have been innocently infected by others who thought themselves cured.

In view of its great frequency, its tendency to become chronic, the serious effects of the chronic form on the individual and the race, and the uncertainty of cure, gonorrhea must be regarded as fully as serious a disease as the more greatly feared syphilis.

At one time, gonorrheal infection of the eyes of infants (*ophthalmia neonatorum*) was very common, but since the universal adoption of the Credé method of prophylaxis,—the application of silver nitrate solution to the eyes of all newborn babies immediately after birth,—this disease has become rare.

In young female children there may occur a troublesome, but not very serious, inflammation of the vulva and vagina due to gonococci. This does not usually spread to the internal genital organs, and although the infection is resistant to treatment, it eventually disappears without resulting in any permanent harm, when the mucous membrane of the vagina becomes matured. The importance of this disease lies in the ease with which it may spread among the children in a hospital or institution. Once a case appears in a children's hospital ward the most careful attention to the sterilization of the patient's towels and other linen, and the most *conscientious practice of hand disinfection by the nurses,* are required to prevent the spread of the infection to other children. In many children's hospitals, vaginal smears are made from every female child on entering the hospital. These smears are examined by the bacteriologist for gonococci, and if any suspicious organisms are found, the child is put under the most strict isolation at once.

Bacteriological diagnosis. A variety of procedures are used to determine the presence of gonorrheal infection. These include: (1) *direct smears,* (2) *cultures,* and (3) *immunological tests* (complement fixation tests) *with the patient's blood. Skin tests* are also under trial.

Direct smears. In *acute cases,* when the pus is abundant, the laboratory diagnosis of gonorrhea usually is not difficult. Smears are prepared from the urethral discharge and stained by Gram's method. If Gram-negative diplococci of the typical biscuit-shaped appearance are found grouped within the pus cells this is sufficient evidence for all practical purposes that the infection is due to gonococci.

In the *male,* the diagnosis is easily made, because few other bacteria are present in the pus. For this reason smears are sometimes stained with methylene blue only, but this is not to be recommended, because it is necessary to show that the cocci are *Gram-negative* before they can be recognized as gonococci with certainty.

In the *female,* the diagnosis is more difficult, because numerous other bacteria, including some diplococci which look very much like gonococci, are usually present. The bacteriologist is sometimes unable to decide definitely whether gonococci are present or not, on the basis of the smear alone, and without cultures or other evidence, can only say "suspicious for gonorrhea." But in most acute cases, in either the male or the female, the germs are readily recognized microscopically and the diagnosis is unmistakable.

In *chronic gonorrhea,* however, diagnosis by means of smears is difficult and may be impossible. The germs are not abundant and there may be no pus, so that it may not be possible to find the gonococci in their typical intracellular arrangement. In the male, it is usually necessary to massage the prostate and seminal vesicles to obtain secretion for examination. In the female, smears are made from the cervix and other limited areas most likely to harbor the organisms.

Cultures. Cultivation of the gonococci from the patient is a supplemental procedure of value in both acute and chronic cases and not infrequently will permit a definite diagnosis of gonorrhea when direct smears are doubtful or negative. A variety of special media have been devised for this purpose. One of the best is a *chocolate agar* medium, made of infusion agar plus 10 per cent of blood which has been heated just enough to make it turn brown. The medium must be moist and previously warmed to body temperature. It should be heavily inoculated, and it is probably best to incubate the cultures in a jar in an atmosphere of 5–10 per cent CO_2.

After growth appears the colonies of gonococci may be quickly found by use of the *oxidase reaction.* This consists in pouring over the medium a sterile 1 per cent solution of tetramethyl-p-phenylenediamine hydrochloride, which is then drained off immediately. By action of the enzyme oxidase, possessed by the organisms, gonococcus colonies quickly turn a pinkish-purple color and finally become black. The organisms are not killed at once by this reagent, and transplants from the reacting colonies may be made successfully within a half-hour's time. The general character of these colonies, the morphology of the organisms found on microscopic examination, and the cultural requirements and fermentation reactions of the cocci as determined in subcultures, will definitely identify them as gonococci.

Complement fixation test. As additional evidence of gonorrheal infection, or as a diagnostic procedure when smears and cultures fail, particularly in those cases in which the germs have set up arthritis or other complicating infections in the internal organs, a *blood test* similar in principal to the Wassermann test used for the diagnosis of syphilis may be performed. This is a complement fixation test in which the patient's blood is mixed with an antigen consisting of a suspension of several different strains of gonococci.

Skin tests for diagnosis are still in the experimental stage.

Immunity. It is doubtful whether recovery from gonorrhea leaves any degree of immunity at all, but, in any case, it is only of short duration, for in a brief period a second attack may be acquired as readily as the first.

Good results are reported in the treatment of gonorrhea with sulfanilamide.

SYPHILIS

This is a specific germ disease, entirely distinct from gonorrhea. It is caused by a kind of spirochete, called *Treponema pallidum.* This organism was first recognized as the cause of syphilis by Schaudinn and Hoffmann in 1905.

Like gonorrhea, syphilis occurs naturally only in human beings. It is possible, however, to produce the disease in apes and in rabbits by artificial inoculation of material from cases of human syphilis.

The origin of syphilis is not definitely known, but it is said to have been introduced into Europe for the first time at the end of the fifteenth century by the sailors of Columbus on their return from their first voyage to America. The disease spread very rapidly throughout Europe. It was at that time a very acute, severe infection, but after centuries, during which it has occurred continually throughout the world, it has now become a milder, chronic disease. The curious name "syphilis" has its origin in a famous poem written by Fracastoro in 1530, in which a character named *Syphilus* is described as having the symptoms of the disease.

Treponema pallidum. *Morphology and staining.* The general properties of spirochetes of the genus *Treponema* have already been described (Chapter VII). The *Treponema pallidum* —the *pale* treponema—is an extremely delicate filament about 10 microns long, with sharp, closely set, and regular spirals. The ends taper to a fine point (Figs. 18, 79). This organism is able to swim backward or forward and swing its body from side to side, while at the same time it rotates on its own axis, like a corkscrew. It is not hard to imagine how such a germ bores its way into the tissues. The organism is difficult to stain, and in smears prepared in the ordinary manner and colored with the usual stains it will not be seen at all. Some of the special methods used to demonstrate spirochetes in smears are mentioned below, and in Appendix B. The syphilis spirochete is best observed in the living state under a *dark-field microscope.*

Physiological properties. In 1911 Noguchi reported that he had succeeded in cultivating *Treponema pallidum* in pure culture and with these cultures had reproduced syphilitic lesions in animals. He used especially enriched, semisolid agar media and anerobic methods. Other investigators, however, have not been able to confirm his results entirely, and there is considerable doubt whether the truly *virulent* syphilis spirochetes have ever been isolated in pure culture.

Because of this uncertainty and the many technical difficulties encountered in its study, there is still much to be learned about this most important organism. It is clear that the syphilis spirochetes are extremely delicate microbes, and very closely adapted to a parasitic life on the human body. They are killed rapidly by cold, or moderate heat, by drying, by weak disinfectants, and even by soap and water. They cannot live in the presence of air. These are fortunate circumstances, for if it were otherwise, syphilis would be even more common than it now is.

The sensitiveness of the spirochetes *within the body* to mercury and arsenicals (salvarsan) is the basis of the successful chemical treatment of syphilis. The fact that in infected animal tissue they are destroyed within an hour when exposed to a temperature as low as 41° C. furnishes the rational ground for the fever therapy now being tried extensively.

How syphilis spreads. On account of the lack of resistance of the germ outside of the body, syphilis is rarely acquired in any other way than by actual bodily contact with the lesions of the disease in another person, though not necessarily through sexual intercourse. The infection may be transmitted by kissing a person who has a syphilitic sore in the mouth or throat. The spirochetes have the power of invading the body through very minute breaks in the skin or mucous membranes. It is not uncommon for nurses or doctors to acquire the disease as the result of infecting a finger during the examination or care of a patient.

It is possible for the disease to be transmitted through the common use of towels or drinking cups, but the chance of infection in this way is small. There is practically no danger in handling specimens of blood from syphilitic patients.

Syphilis is often transmitted by a syphilitic mother to her children (Fig. 79). The transmission is not by direct inheritance, and the spirochetes cannot have come directly from the father, but the mother herself must be infected, even though outward symptoms of syphilis may be entirely lacking. The germs probably reach the fetus by growing through the placenta. The result may be the death of the baby (still births are far more common in syphilitic families than in nonsyphilitic families), the baby may be born with active signs of the disease, or the infant may show no evidence of the disease at birth, but symptoms corresponding to the late stages of syphilis may develop several years later. Not all children of mothers with latent syphilis are necessarily infected. If the mother is treated with salvarsan during pregnancy the danger of infecting the growing baby is practically eliminated.

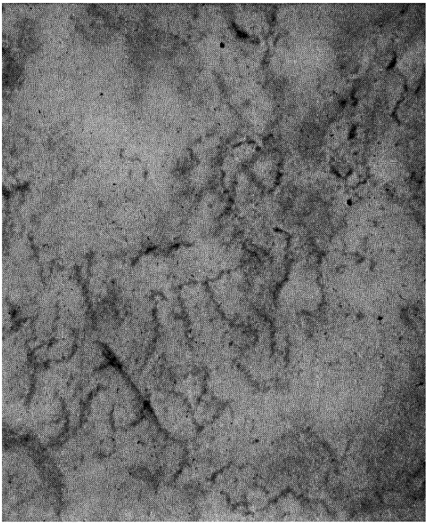

FIG. 79. The syphilis spirochete (*Treponema pallidum*) in a section of the intestine from a syphilitic fetus. The tissue is literally swarming with the spirochetes. The intestine is an unusual site for a congenital syphilitic lesion. The organisms are most commonly demonstrated in the liver, adrenal, heart, and pancreas, although they are present in virtually every body tissue. (Photograph reproduced through the courtesy of Dr. Rigney D'Aunoy and Dr. Bjarne Pearson.)

Clinical forms of syphilis. The first sign of syphilis is the appearance of a sore called a *chancre*, at the spot where the spirochetes have entered two to five weeks before. The chancre is not always typical in appearance and it may be hidden so that it is overlooked. This primary sore literally swarms with the syphilis spirochetes.

Whether or not the proper treatment is given, the chancre soon disappears. (This makes possible the evil profits of the quack doctor). However, serious trouble is just beginning, for the spirochetes are now invading the entire body. In about a month, or longer, the so-called secondary stage of the disease is established. This is characterized by a skin eruption, by sores in the mouth or throat, and by other evidences of an involvement of the entire body. At this secondary stage the patient is a source of great danger for others, and syphilis has often been acquired innocently by contact with such persons.

After the acute symptoms of the secondary stage have passed, there follows a period of variable length, often months or years, in which the patient is practically without symptoms, though there may be relapses from time to time. The destructive effect of the germs still present in the body becomes obvious at last, however, with the development of symptoms, which may be of the most varied nature and may simulate the symptoms of many other diseases. In about a third of the cases, not effectively treated in earlier stages, the brain and spinal cord may finally become the seat of destructive lesions, resulting in locomotor ataxia (tabes dorsalis), general paresis, insanity, and death. During this third stage of syphilis, the patient is probably not able to transmit the infection to others.

Syphilis is one of the very few diseases for which we have a *specific* chemical remedy. This is the arsenical preparation called *salvarsan* or *arsphenamine*, first made by Ehrlich in 1909. This drug kills the spirochetes in the body and, when given in sufficient amount in the earlier stages of the disease, will bring about a complete cure. Mercury and other chemicals are also given as part of the treatment. In most cases it is necessary to continue the treatment for two years or more.

Fever therapy, employed in connection with the usual chemical treatment, has resulted in remarkable clinical improvement in cases of syphilis, especially in advanced syphilis of the central nervous system (paresis). Fever is induced most commonly by producing in the patient an attack of *malaria* by inoculating him with blood containing malaria parasites. This artificially induced infection can be readily controlled by quinine. Various machines for the direct heating of the body have been devised and these are finding increasingly wide use in fever treatment.

Bacteriological diagnosis. A laboratory diagnosis of syphilis may be made in two ways: (1) by microscopic examination of fluid from the chancre, or (2) by immunological tests with the patient's blood serum or spinal fluid.

Dark-field examination, and smears of chancre fluid. If the typical spirochetes are found in the primary sore, this affords the earliest and surest proof that the patient has syphilis. Negative results are not entirely conclusive, however, because this microscopic examination of the chancre is somewhat difficult to carry out successfully. The best method is to examine fluid squeezed out of the chancre under the *dark-field microscope,* so that the spirochetes can be seen in the living state. If this cannot be done smears of the fluid may be made by the India ink method, or the nigrosine method, or dried smears may be treated with Fontana's stain or other special spirochete stain (Appendix B).

Blood (and spinal fluid) tests. After the primary stage the laboratory diagnosis of syphilis is made by testing the blood (or, when involvement of the central nervous system is suspected, the spinal fluid), of the patient for the presence of the peculiar antibody formed as the result of syphilitic infection. Blood tests become positive two to three weeks after the appearance of the chancre. The best known of the various blood tests for syphilis is the *Wassermann test,* originally devised by Wassermann and Bruck (1906). This is a *complement fixation test,* the principle of which has already been explained (p. 327). The Wassermann test is occasionally positive in conditions other than syphilis, such as malaria and leprosy, and in some circumstances may be negative in syphilitic individuals, but in general, it gives reliable results, and when taken in connection with clinical signs and history, is of immense value as an aid to diagnosis and as a guide to treatment. The test is complicated, however, and subject to many errors. In all cases with doubtful clinical signs, repeated tests should be made before a positive diagnosis is accepted.

In recent years a number of other tests, simpler than the Wassermann test, but equally reliable when properly performed, have been developed. Those most widely used in this country are the Kahn test and the Kline test. These are called "precipitation tests," because a visible precipitate or clumps are formed in positive cases when the blood serum of the syphilitic patient is mixed directly with the antigen preparation.

The same methods may be used for testing the spinal fluid. Sometimes the blood test is negative while the spinal fluid gives a positive reaction.

As the patient recovers following treatment, the Wassermann and precipitation tests become *negative*. This is in sharp contrast to the usual state of affairs in other diseases. Ordinarily the immunological tests we perform for diagnostic purposes on the patient's blood in other infections are most strongly positive at the time of recovery and shortly thereafter, indicating an abundant content of protective antibodies at that time. But in the tests for syphilis we detect an antibody not related to resistance, but associated in some way with the presence of spirochetes in the body. A positive Wassermann (or precipitation) test is then, *a sign of syphilitic infection*, not an index of immunity, and naturally this sign disappears as the spirochetal infection is conquered.

Immunity. Antibodies which will act directly upon the syphilis spirochetes may be demonstrated in the blood of syphilitic patients, but resistance to the infection seems to depend principally upon some obscure cellular change which makes the tissues unfavorable for the organisms. This change develops during the course of syphilis and it is a well established fact that as long as a person is obviously infected with the syphilis spirochete, *reinfection* with this germ is very rare. A full recovery from the disease, however, does not leave any effective immunity, for reinfection may now occur, and the individual appears to be as susceptible as ever.

GRANULOMA INGUINALE

Prevalence and clinical features. This infection (also called granuloma venereum) was formerly regarded as almost exclusively a tropical disease, but in recent years it has been shown to be endemic in the United States, and by no means uncommon, particularly among the poorer classes of the colored race in the South. Beginning as a small, moist papule on or near the external genitalia, the infection gradually creeps into the surrounding mucous membranes and skin, and may eventually become a large superficial ulcerating lesion, involving a considerable area of the groin. The disease remains localized to the pubic region, and despite severe and extensive lesions there, the patient usually shows little or no signs of general illness. The causative agent of granuloma inguinale is presumably transmitted by sexual contact, but the disease is not highly contagious and may possibly be acquired in some other way. Remarkable clinical improvement occurs in most cases following intravenous injections of tartar emetic.

Etiology and diagnosis. The true cause of granuloma inguinale is an unsettled question. Smears made from the ulcerating lesions, reveal large mononuclear cells (macrophages) containing numerous round or oval bodies, called *Donovan bodies.* Laboratory diagnosis of the disease is now based upon the finding of these characteristic cells with the typical "bodies" in the cytoplasm. The nature of the latter is uncertain (Fig. 80).

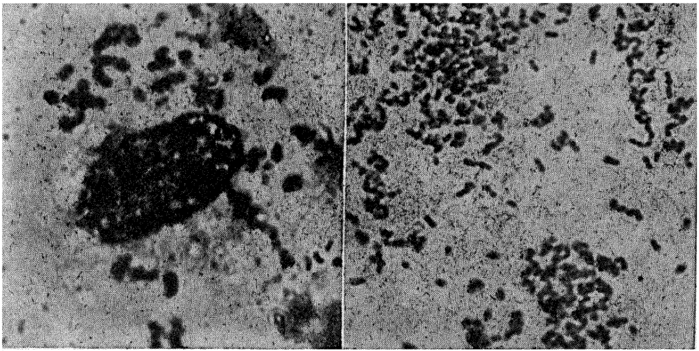

FIG. 80. Left, Donovan bodies within the cytoplasm of a large mononuclear cell in a direct smear, stained by Wright's method, from the skin ulceration of granuloma inguinale. The large, oval body is the nucleus of the cell. The Donovan bodies often appear relatively much smaller. Right, smear of the capsulated Gram-negative bacilli which may often be cultivated from granuloma inguinale lesions. The relation of these cultivable bacteria to the Donovan bodies and to the etiology of the disease is uncertain.

FUSO-SPIROCHETAL INFECTION

The same combination of micro-organisms responsible for the ulceration in the throat known as Vincent's angina (Fig. 68) may cause similar ulcerating lesions of the external genitalia in both male and female. The bacteriological diagnosis is readily made by microscopic examination of direct smears from the lesions, stained by gentian violet or a special spirochete stain. Treatment with salvarsan often proves effective.

LYMPHOGRANULOMA INGUINALE

Prevalence and clinical features. This is the disease known in the tropics as *climatic bubo*, and also called lymphogranuloma venereum, or the sixth venereal disease. For a long time confused with other diseases of the genitalia, it is now known to be a specific infection caused by a *filtrable virus.* The student should note particularly that *lymphogranuloma inguinale* is a separate and distinct infection from the granuloma inguinale described above.

Though long known as a tropical disease, lymphogranuloma inguinale has been recognized only in comparatively recent years as an infection of wide occurrence in temperate regions as well. It is prevalent among the colored population in the southern United States.

There is probably a small initial sore, as in syphilis, but usually this cannot be found. In the male, there soon occurs a swelling of the inguinal lymphatic glands, forming the typical bubo. In the female it is the more deeply situated, intrapelvic lymphatic glands that are the usual site of active infection. In consequence, there is often considerable tissue-destruction and scarring, one common effect of which is to produce a stricture of the rectum. Unlike granuloma inguinale, which is a localized infection confined to the genital region, lymphogranuloma inguinale may be generalized.

The virus of lymphogranuloma inguinale. From the swollen inguinal glands, and other lesions, bacteria-free pus may be aspirated, filtrates of which have been shown to contain the specific virus of the disease. Inclusion bodies and elementary bodies have been described. The size of this virus has been estimated to be about the same as that of the virus of smallpox, *i.e.*, the particles are about 150 mμ in diameter. Several animal species, including monkeys and mice are susceptible to experimental infection and the virus is usually maintained in laboratories by serial transfer intracerebrally in white mice.

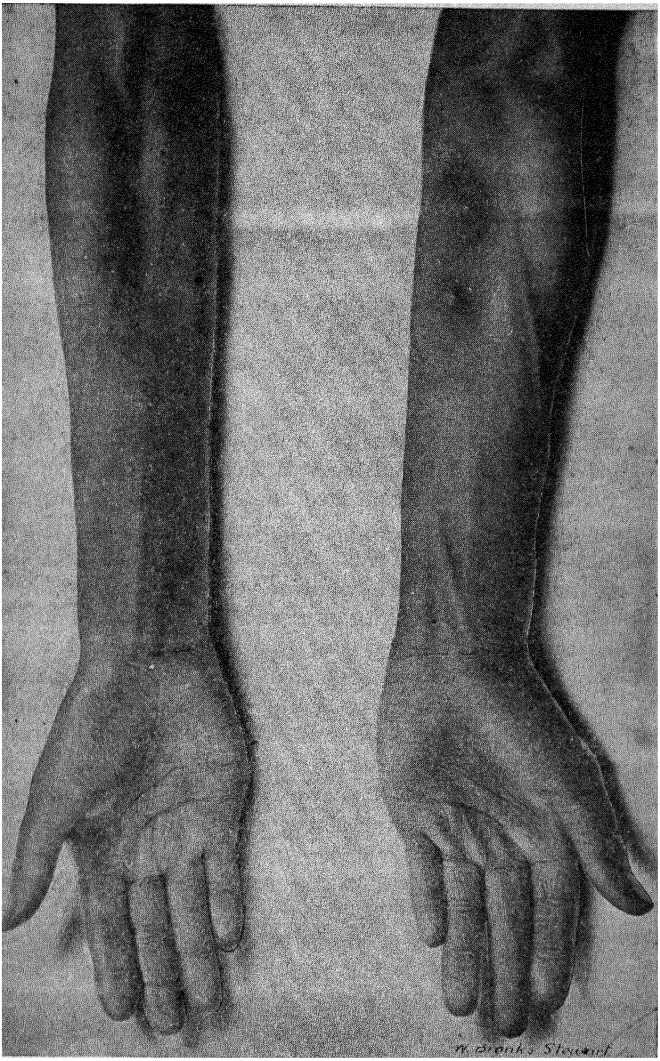

FIG. 81. **Right,** a positive Frei test (with each of two antigen preparations); left, the opposite arm showing no reactions from control injections. (Reproduced from a painting by W. Branks Stewart; courtesy of Dr. Rigney D'Aunoy and Dr. Emmerich von Haam.)

Diagnosis; the Frei test. Frei in 1925 found that an inflammatory (allergic) skin reaction is produced in lymphogranuloma inguinale patients when a minute amount of the diluted, heated pus from the swollen inguinal lymph gland of another patient is inoculated intradermally (Fig. 81). At the present time the Frei test is widely used for diagnosis. The material to be injected is prepared either from infected human glands or from the brains of experimentally infected mice. In any case it is heated so that it contains no active virus. The results of the test must be interpreted, of course, in the light of the clinical history of the patient.

PREVENTION OF VENEREAL DISEASES

Prevention of syphilis and gonorrhea is generally admitted to be a public health problem of outstanding importance. It is partly a social problem and partly a medical problem, and obviously a difficult and complicated one. Efforts to improve social conditions in general, and particularly measures to suppress prostitution, which is, after all, the real root of this great evil, will eventually reduce the amount of venereal disease. The establishment of clinics for diagnosis and treatment, and hospital beds for the isolation of cases in the communicable stages, discovery of cases by routine examination of food handlers, employees in industrial plants, etc., and persons in public institutions of all kinds, reporting of all cases by physicians to the health authorities, and especially, *tracing cases to their source* by investigations of trained nurses or social workers are among the principal measures which are now being tried in nearly every large community. Authorities will be greatly aided in their efforts when the public as a whole realizes that the control of these diseases is primarily a public health problem, not a moral problem. A great advance has been made in this direction since 1937, when the United States Public Health Service began the present nationwide antisyphilis campaign.

The really effective preventive, in the long run, is the proper education of everyone on this subject. A great step forward will be made when every individual who has a suspicious sore or discharge, especially if he has knowingly exposed himself, realizes that the best thing he can do for himself, as well as for everyone else, is to submit at once to a thorough examination by a competent physician, and if found diseased to accept at once the necessary course of treatment. Early diagnosis and adequate treatment in syphilis are especially important and, in fact, if every case of syphilis could be discovered in its earliest stages and intensively treated by trained physicians, the patients *would quickly be rendered noninfectious*, the disastrous effects of advanced syphilis would be avoided, and this disease would very soon cease to be the menace it now is.

REVIEW QUESTIONS—CHAPTER XXXIV

1. What organisms may be present on the external genitals?
2. Describe common infections of the urinary tract and the organisms usually concerned.
3. How is a bacteriological diagnosis of cystitis or pyelitis made?
4. What is puerperal fever? What is the cause and origin of severe and fatal cases?
5. What did Holmes and Semmelweis contribute to early knowledge of the nature of puerperal fever?
6. What is the origin of a form of puerperal infection common at the present time? What will help to prevent this infection?
7. Name the three common specific infections of the genital organs. Name three other, less prevalent, specific infections, primarily involving the the genital region. Discuss the prevalence and importance of gonorrhea and syphilis.
8. Describe chancroid. Name and describe the causative organism and outline practical methods of bacteriological diagnosis.
9. Name the organism causing gonorrhea. When was it discovered and proved to be the cause of this disease and by whom?
10. Describe morphology and staining of gonococci and their appearance in smears of pus.
11. Describe the principal physiological properties of gonococci. What resistance do they have outside the body?
12. Describe the clinical forms of gonorrhea in adults. What serious effects may the disease have on the individual and on the race?
13. What form of gonorrheal infection was formerly common in infants? How is this now prevented?
14. What form of gonorrheal infection may occur in young female children? What precautions must be taken to prevent its spread?
15. How is a bacteriological diagnosis of gonorrhea made (*a*) in acute cases, (*b*) in chronic cases? Discuss the value and the limitations of (1) smears, (2) cultures, (3) serological tests.
16. Is there any immunity to gonorrhea?
17. Name the causative organism of syphilis. Who discovered the organism and when?

18. When was syphilis first introduced into Europe? Who gave the disease its name?
19. Describe the *Treponema pallidum*. How can it be examined microscopically? How can this organism be cultivated? What is known of its resistance outside the body? Within the tissues?
20. How may syphilis be acquired?
21. Describe briefly the three clinical stages of syphilis. Are they equally dangerous to others?
22. In what two ways may a laboratory diagnosis of syphilis be made? What is the value of microscopic examinations?
23. What is the principle of the Wassermann test? Discuss its value and limitations. Name two other blood tests recently developed.
24. What can be said concerning immunity to syphilis?
25. Characterize the infection called granuloma inguinale. How is the laboratory diagnosis made?
26. What is meant by fuso-spirochetal infection of the genitalia? How is the bacteriological diagnosis made?
27. Describe the outstanding clinical features of lymphogranuloma inguinale. What is the nature of the etiologic agent? Distinguish between granuloma inguinale and lymphogranuloma inguinale.
28. Describe the properties of the virus of lymphogranuloma inguinale.
29. Describe the materials and procedure used, and the proper interpretation of the Frei test.
30. Discuss the problem of the prevention of venereal diseases.

CHAPTER XXXV

INTESTINAL INFECTIONS AND INTOXICATIONS. FOOD POISONING. BOTULISM

INTESTINAL INFECTIONS AND INTOXICATIONS

THE INTESTINAL tract, which is some 30 feet long, may be thought of as a tube which is within the body, and yet is really outside of the body proper. It contains continuously a remarkably varied and abundant flora of micro-organisms. In fact, as a culture medium and incubator combined, the intestine has no rival in Nature. In the colon (large intestine) especially, a tremendous number of organisms are to be found at all times, and a very large proportion of the bulk of the intestinal discharges (feces) is composed of nothing more than the bodies of bacteria, the majority of which are dead. The enormous number of bacteria in the feces is clearly indicated by the appearance of direct smears (Fig. 82). Obviously, the food we swallow does not contain anything like this number of bacteria; therefore, there must be a very rapid multiplication of the organisms within the intestine.

Normal intestinal flora in infancy and in adult life. The intestinal canal of the new-born baby is sterile, but within a few hours after birth, bacteria gain entrance through the mouth and anus from objects in the immediate surroundings. During the first two or three days of life, the feces of the infant will contain the various bacteria that have accidentally found their way into the intestine, but by the fourth or fifth day, when the infant has begun to take milk regularly, these organisms largely disappear, and a peculiar bacterial flora characteristic of the infant intestine becomes established. The striking thing about this flora is its remarkable simplicity and uniformity, particularly in the case of infants who are breast-fed. In most cases, the feces of the breast-fed infant contain not more than three or four kinds of organisms, and as much as 99 per cent of all the bacteria may be of a single kind—the anaerobic bacillus, called *Lactobacillus bifidus.* Smears made directly from the breast-fed infant feces show in a striking way the great preponderance of this organism (Fig. 83). The few other bacteria most likely to be present include a Gram-positive streptococcus, called *Streptococcus fecalis* (or enterococcus), *Lactobacillus acidophilus,* and *Escherichia coli* and related Gram-negative bacilli. The latter, however, are always in small numbers as compared with their abundance in the adult intestine.

In the case of bottle-fed infants, the intestinal flora is likely to be much more varied, with fewer *Lactobacillus bifidus,* and more of *Escherichia coli* and other organisms characteristic of the adult intestine, including aerobic spore-bearing bacilli, and *Clostridium welchii* and other anaërobes. However, if the infant is fed soured milk, that is, *acid* milk, almost the same type of intestinal flora will develop as in the breast-fed child.

The *Lactobacillus bifidus* is so called because in broth cultures from the feces it shows characteristic forked (bifid) ends. This organism forms considerable acid from the sugar (lactose) in milk, and its presence in such large numbers in the infant intestine tends to inhibit the development of the proteolytic type of bacteria, which are, in general, sensitive to acid and unable to multiply in an acid environment. Thus, so long as the infant is receiving an exclusive milk diet and this acid-producing flora predominates, protein-splitting organisms are kept out, the feces do not have a putrefactive character as in the adult, and the possibility of the absorption from the intestine of toxic substances formed there by proteolytic bacteria is avoided.

As the individual grows older, and milk is no longer the sole or the principal article of diet, the intestinal flora becomes more and more complex, until, in the adult, organisms of many different kinds become abundant. *Escherichia coli* and related Gram-negative bacilli—the group of organisms generally referred to as the *colon bacilli*—come to be the predominant bacteria in the lower intestine, and they constitute about 75 per cent of all the living bacteria in the feces.

Bacteria are not uniformly distributed throughout the length of the intestine, but there are differences in the number and kinds of organisms at different levels. The empty stomach is usually sterile, and there are very few organisms in the duodenum and upper jejunum. This is probably due to the acid secretion of the stomach. The lower levels of the small intestines become progressively richer in bacteria, and in the large intestine the number of organisms reaches the maximum. Here, besides colon bacilli, staphylococci, sarcinae, and yeasts, are found aerobic spore-forming bacilli, such as *Bacillus mesentericus* and *Bacillus subtilis*, anaerobic spore-bearers, such as *Clostridium welchii*, aciduric bacteria, such as *Lactobacillus acidophilus*, thermophilic bacteria, spirochetes, and various other types.

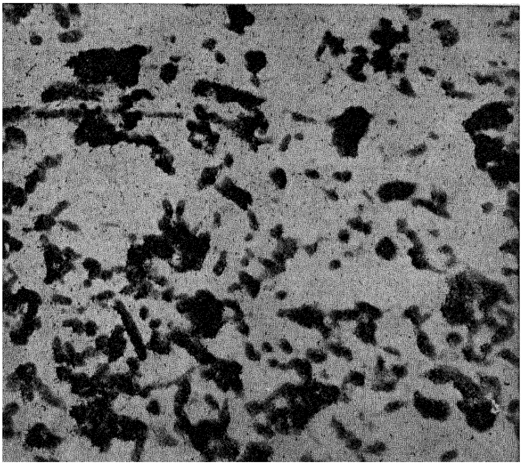

FIG. 82. Direct smear from the feces of an adult. The smear shows large numbers of bacteria of many different kinds. Compare with Figure 83.

Importance of bacteria in the intestines. Influence of diet upon the intestinal flora. The masses of organisms in the intestines under normal conditions do no harm, neither do they benefit their host to any appreciable extent. They probably do not play any essential part in the digestion of food, though perhaps they do assist somewhat in this process.

The normal human adult, living on a balanced dietary, will have in the intestines a mixed flora which will not be dominated by either proteolytic or fermentative types of organisms. But if the diet, day after day, contains an excess of protein, and particularly if at the same time there is faulty elimination, proteolytic bacteria may become abnormally abundant, and toxic products, formed as a result of their activity, may be absorbed from the intestine, and as a consequence the general health may be more or less seriously affected. It is in such cases that "sour milk therapy" has been found to be helpful. If the individual will drink milk soured with *Lactobacillus acidophilus* and at the same time will take large amounts of lactose daily, it is possible to implant this aciduric organism in the lower intestine, and encourage its growth at the expense of the proteolytic organisms, with the result that constipation and other clinical symptoms are relieved.

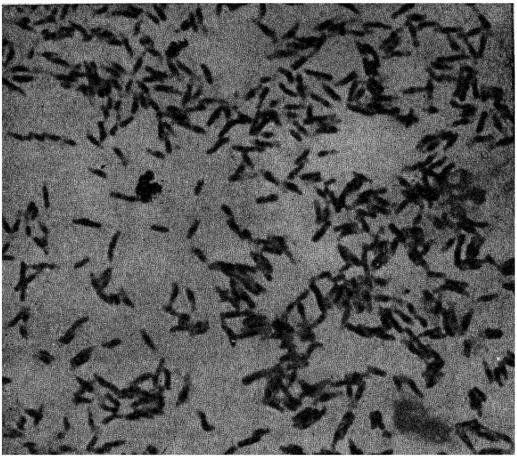

FIG. 83. Direct smear from the feces of a five-day-old breast-fed infant. Note that bacteria are abundant as in adult feces (Fig. 82), but are almost all of a single kind. They belong to the species *Lactobacillus bifidus*. Note forked (bifid) form near upper right corner of photograph.

The colon-typhoid-dysentery group of bacilli. Despite the great complexity of the bacterial flora in the intestine, only a few kinds of organisms need be considered by bacteriologists in connection with common infections of the intestinal tract. We have already mentioned that a large proportion of the living bacteria in the feces belong to the species called *Escherichia coli*, or to one of the related species constituting the colon group. The numerous varieties of colon bacilli are all Gram-negative, without spores, and morphologically indistinguishable from each other. It happens that the germs of typhoid fever, paratyphoid fever, some forms of food poisoning, and dysentery—that is to say, the organisms which cause all the common acute infections of the intestinal tract (except the germ of cholera, which is a spirilum)—are also Gram-negative, nonspore-bearing bacilli, having the identical appearance and differing only slightly in other ways, from the colon bacilli of the normal intestine. Thus, all of these organisms, pathogenic and nonpathogenic, may be regarded as forming a single group of closely related species—the colon-typhoid-dysentery group.

In the following table the principal members of this group, and the most important of the properties which help to differentiate them, are given. The list includes by no means all of the known varieties of intestinal bacilli, but only those of greatest importance.

NAME OF ORGANISM	MOTILITY	FERMENTATION OF				GELATIN LIQUEFACTION	INDOL PRODUCTION	H_2S PRODUCTION
		DEXTROSE	LACTOSE	SACCHAROSE	MANNITE			
Escherichia coli (B. coli communis)	+	ag	ag	—	ag	—	+	—
Escherichia communior (B. coli communior)	+	ag	ag	ag	ag	—	+	—
Proteus vulgaris (B. proteus)	+	ag	—	ag	—	+	+	+
Alkaligenes fecalis (B. alcaligenes)	+	—	—	—	—	—	—	—
Eberthella typhi (B. typhosus)	+	a	—	—	a	—	—	+
Salmonella paratyphi (B. paratyphosus alpha)	+	ag	—	—	ag	—	—	—
Salmonella schottmülleri (B. paratyphosus beta)	+	ag	—	—	ag	—	—	+
Salmonella enteritidis (B. enteritidis)	+	ag	—	—	ag	—	—	+
Shigella dysenteriae (B. dysenteriae, Shiga)	—	a	—	—	—	—	—	—
Shigella paradysenteriae (B. dysenteriae, Flexner)	—	a	—	—	a	—	+	—

NOTE: a = fermentation with production of *acid.*
ag = fermentation with production of *acid and gas.*

When it is remembered that all the organisms listed in this table have practically an identical microscopic appearance and staining reaction, it is clear that they belong to the same family of microbes, and the bacteriologist must use special care in order to identify positively any one of them. All are *motile*, except the dysentery bacilli. All of them grow readily on plain culture media and their manner of growth, character of agar colonies, etc., are very similar. The most important difference in fermentation reactions is that *lactose is fermented only by the colon bacilli*, and not by the typhoid, paratyphoid, or dysentery bacilli. This fact is the basis for the practical methods used to isolate these pathogenic organisms from the feces, for the purpose of diagnosis. However, fermentation reactions, using the carbohydrates given here, would not serve to identify any of these pathogens definitely, for it will be seen that the typhoid and dysentery bacilli (Flexner variety) both give the same reactions (acid only in dextrose and mannitol), and the varieties of paratyphoid organisms also ferment the same carbohydrates, in this case with acid and gas. *The final identification of any one of these intestinal organisms depends upon agglutination tests with specific immune serum.* Thus, proof that a bacillus is really *Eberthella typhi* can be secured positively only when it is shown that the bacillus will agglutinate in high dilutions of a serum containing specific antityphoid agglutinins.

Escherichia coli and *Escherichia communior* are the most abundant of the living organisms in the feces of animals as well as man. The student will recall that the organism is used as the "index of pollution" in connection with the bacteriological analysis of water. Whenever it is abundant in a sample of drinking water, this is evidence that the water has been polluted with intestinal material. The organism lives quite harmlessly in the intestine, but it has some disease producing powers, and in this respect is a typical "opportunist." It is one of the bacteria most commonly causing inflammation of the urinary tract (cystitis, pyelitis), and of the gall bladder (cholecystitis). It is also abundant in many cases of appendicitis and peritonitis, and rarely in other inflammatory conditions within the body.

Proteus vulgaris is noteworthy because it possesses considerable power to attack complex native proteins, that is, it is a proteolytic organism. It will form indol and liquefy gelatin. It is the only member of this group of bacteria, except *Escherichia communior*, that ferments saccharose. Numerous varieties of this organism are found naturally in decomposing materials, and they also occur frequently in the feces of adults. In the intestine, the proteus bacillus ordinarily does no harm. But, like the colon bacillus, it is an "opportunist," and is frequently associated with rather serious inflammations. It is thought to be the cause of some cases of acute food poisoning, and of summer diarrhea in children. It is also found in such conditions as gangrenous wounds, appendicitis, peritonitis, otitis media and inflammations of the urinary tract.

Alkaligenes fecalis is a common inhabitant of the intestine which has occasionally been held responsible for typhoid-like disease. It is conspicuous among the intestinal organisms, because it fails to ferment any carbohydrates.

Eberthella typhi (old name *Bacillus typhosus*) is the specific cause of typhoid fever.

Salmonella paratyphi and *schottmülleri* (the alpha and beta paratyphoid bacilli), and *Salmonella enteritidis* are the principal representatives of a very large group of closely related so-called "paratyphoid bacilli." These organisms are responsible for various forms of illness in animals, and in man they cause paratyphoid fever and food poisoning.

Shigella dysenteriae and *paradysenteriae* (the *Shiga* and *Flexner* dysentery bacilli, respectively) are the principal varieties among the many strains of similar organisms which may be responsible for bacillary dysentery in man.

Common nonspecific infections of the intestinal tract. *Peritonitis.* The delicate membrane which lines the abdominal cavity and envelops the intestine and other organs there—the peritoneum—is sometimes inflamed, as the result of a primary infection with *Pneumococcus* or other organisms, particularly in children. In most cases, however, peritonitis is secondary to some lesion in the stomach or intestines and follows perforation of a gastric or duodenal ulcer, intestinal obstruction caused by intersusception or hernia, rupture of an inflamed appendix, intestinal operations, or abortions. Though many types of bacteria may be present, the peritoneal exudate usually contains a predominance of *Streptococcus hemolyticus* or *Escherichia coli.*

Cholecystitis. Inflammation of the gall bladder is commonly caused by colon bacilli, sometimes associated with streptococci. The presence of gallstones probably greatly increases the liability to gall bladder infections, by causing irritation, and by obstructing the free flow of bile, and thus making more likely the passage of bacteria up the bile ducts to the gall bladder from the intestine. Doubtless, the gall bladder is at times infected by organisms brought to it in the blood, rather than directly from the intestine.

Appendicitis. The appendix in man is a small, closed tube about four inches long and as large around as the little finger, attached as a sort of wormlike appendage to the large intestine just below the junction with the small intestine (Fig. 84). In human beings, the appendix is a useless organ—an evolutionary remnant—but in certain herbivorous animals, rabbits for example, a corresponding structure, much larger, serves as a pouch in which partial digestion of the food takes place.

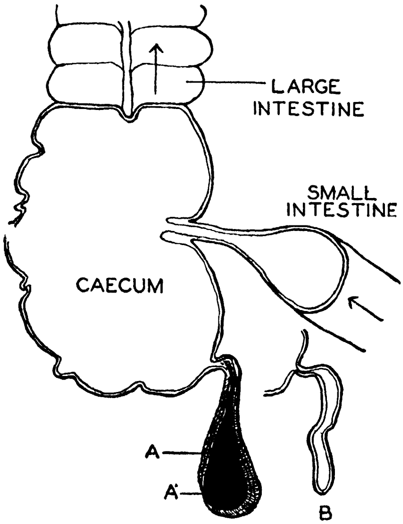

Fig. 84. Diagram of portion of the intestine at the junction of the small and large intestine, cut open to show the position of the appendix. The arrows indicate the direction in which the food passes through. B, the normal appendix. A, the condition in acute appendicitis. The appendix is swollen and filled with pus; perforation is about to occur at A'.

Both acute and chronic infection of the appendix are common. In the acute cases, a rapidly developing inflammation with formation of pus takes place. Usually the only adequate treatment is the immediate surgical removal of the inflamed organ. If this is done before the wall of the appendix becomes gangrenous and perforation occurs, an immediate cure is effected, otherwise there may develop a localized peritoneal inflammation, or a fatal generalized peritonitis.

A mixture of many different organisms of both aerobic and anaerobic types may be found in cases of acute appendicitis. Among the aerobic bacteria likely to be present are *Escherichia coli*, *Proteus vulgaris*, staphylococci, and streptococci. Anaerobic organisms are usually abundant, especially when the infection is of a severe, gangrenous character. Among these anaerobes may be *Clostridium welchii* and other Gram-positive bacilli of the types common in gangrenous wounds, *Bacterium melaninogenicum*, and the Gram-negative, nonspore-bearing *fusiform bacilli.*

FOOD POISONING

So-called "ptomaine poisoning." Food poisoning of the type popularly called "ptomaine poisoning" is characterized by the sudden development of nausea, vomiting, diarrhea, and abdominal pain within about twelve to twenty-four hours after a meal. The illness usually passes in a day or two, but may be fatal. As we have already pointed out, *ptomaines* (which are mildly toxic substances formed during the latter stages of protein decomposition) *probably have nothing whatever to do with this disease, and the term "ptomaine poisoning" should be given up.* It would be much better to speak of this illness as *bacterial food poisoning,* because there is certain evidence that the disease is due to an infection of the intestinal tract by certain bacteria from the food and to an intoxication caused by poisons formed in the intestines by these organisms.

The bacteria most commonly associated with food poisoning belong to the group of "paratyphoid bacilli." Most often, the species called *Salmonella aertrycke* or *Salmonella enteritidis* is responsible. Other varieties of this genus and other types of intestinal bacilli, have occasionally been incriminated. In some recent cases *Staphylococci* have been shown to be the cause.

In most outbreaks of food poisoning due to paratyphoid bacilli, the food concerned is meat, milk, fish, or other animal protein, and rarely vegetables or fruits. Very often the food is of the kind which has been made up into pies, stews, jellies, sausages, or similar products, and has, therefore, been handled to an unusual extent by the cooks. Also, it often happens that the food responsible for the poisoning is a large dish, prepared to serve a number of guests at a dinner or picnic, and usually such a dish has not been thoroughly cooked. The temperatures reached in ordinary cooking are not so high as is generally believed, and it is easy to see how the center of a large meat pie, for example, might not be heated sufficiently to kill the bacteria present.

In warm weather the danger of food poisoning is especially great, when incompletely cooked food is allowed to stand, without proper refrigeration, some time before it is eaten. Home-canned foods are often responsible for cases of poisoning, as the result of failure to sterilize the food during the canning process. It is important to remember that very dangerous food, heavily contaminated with *Salmonella enteritidis*, for example, may still have an entirely normal appearance and odor.

The germs of food poisoning, especially *Salmonella aertrycke*, sometimes cause infections in cattle and swine, and some cases of food poisoning in man have been traced to the eating of imperfectly cooked meat derived from these sick animals. More commonly, however, meat is contaminated after slaughter of the animal, perhaps by soiling with intestinal contents, or from the excreta of rats and mice which often suffer naturally from infection with paratyphoid bacilli. *In many instances, the source of the contamination of the food is a human carrier of the germ in the person of the cook.* This explains why made-up foods of various kinds are so often responsible for cases of the disease. The outbreaks of *Staphylococcus* food poisoning have been traced most often to cream puffs and similar pastries.

Prevention of this kind of food poisoning requires cleanliness and care during the whole process of securing and preparing food for consumption. A thorough system of meat inspection, sanitary control of bakeries, slaughter houses, and establishments making sausages and similar products, and of public eating places of all kinds, are valuable measures. Meats and other foods should be as fresh as possible and *thoroughly cooked* before they are eaten. In canning, the food should be heated very thoroughly and it is a safe rule to heat again any canned food just before it is served. It is especially important that persons should not be employed as cooks, or in other capacities in which they handle food, who have previously suffered from intestinal infections, and are, therefore, possibly carriers of food poisoning organisms or other pathogenic members of the intestinal bacilli.

Botulism. Another form of food poisoning of entirely different nature, and fortunately rare, is called *botulism.* It is due to the eating of food contaminated with the *Clostridium botulinum* and containing the specific *exotoxin* formed by this organism in the food. The name "botulism" is from the Latin *botulus*, a sausage, and was originally applied to this disease because numerous cases, particularly in Germany, were caused by contaminated sausages. This is by no means the only kind of food which may be responsible, however. In the United States most cases have been traced to canned vegetables.

Clostridium botulinum was first isolated by van Ermengem in 1896 from a piece of raw ham and from the organs of persons who had died of botulism after eating part of this ham. It is an anaerobic, Gram-positive, saprophytic, spore-bearing bacillus, native to the soil. It probably never multiplies to any extent within the body, and botulism is essentially an intoxication, not an infection. The spores of the bacillus are widely distributed and are likely to get upon all kinds of foods. But the eating of fresh foods never causes botulism. Practically all cases can be traced to foods which have been preserved in some way, by smoking, pickling, or canning, allowed to stand for a time, then eaten without cooking, or after only partial cooking. This is explained by the fact that, whereas these methods of preservation destroy most of the organisms in the food, they may not kill all of the resistant spores of *Clostridium botulinum,* and in the interval before the food is eaten, these still-living spores germinate, the organisms then multiply, and in time there accumulates in the food an extraordinarily powerful soluble toxin.

Several cases are known in which deaths from botulism have followed the mere tasting of such food. The botulinus exotoxin has been found to be much more deadly than the strongest tetanus toxin yet prepared. It is peculiar in that it is the only exotoxin formed by bacteria in lifeless materials outside of the living body. It can pass through the walls of the stomach and intestine unchanged, differing in this respect from the toxins of the tetanus and diphtheria bacilli, which are harmless when taken by mouth. It has been found that there are two types of *Clostridium botulinum* which may cause botulism in man, and each of these, Type A and Type B, produces a slightly different toxin, so that antitoxin prepared for one of the types will neutralize the toxin of that type only, and not the other. The majority of cases of botulism in the United States have been caused by Type A.

The symptoms of botulism usually develop within less than twenty-four hours after eating the poisonous food. In contrast to the ordinary type of bacterial food poisoning there are usually no signs of acute gastrointestinal irritation; there is no diarrhea, but instead the patient is constipated. The very distressing symptoms are due to the injurious effect of the botulinus toxin on the nerves. Paralysis of the eye muscles brings on disturbances of vision very early, and soon the paralysis affects the pharynx, the throat becomes clogged with a thick, glairy mucus, and although very thirsty, the patient cannot swallow, and often cannot talk. There is marked general muscular weakness. Usually the patient feels no pain and the mind remains clear. Death is caused primarily by paralysis of the respiratory center. In the United States, about 60 to 70 per cent of the patients die.

Although specific *antitoxins* for the toxin of each of the types of *Clostridium botulinum* are available, it is seldom possible to use them effectively in the treatment of a case of human botulism. The injection of antitoxin of the wrong type would be of no help, and usually there is no way to determine, in time, which of the types of toxin is causing the poisoning.

The toxins of this bacillus are responsible for various forms of poisoning in animals, such as limberneck of chickens, forage poisoning in horses, and paralytic disease in cattle. Cats and dogs sometimes develop botulism.

In recent years, botulism in human beings due to commercially canned food is almost unknown. On the other hand the disease has often been caused by home-canned string beans, corn, or other foods. This emphasizes the importance of selecting for canning only clean, fresh food, and of using the utmost care and thoroughness in the canning process. Thorough cooking of food just before eating is the best safeguard against botulism, as it is against other forms of food poisoning.

REVIEW QUESTIONS—CHAPTER XXXV

1. How do the feces come to contain so many bacteria? Describe the normal bacterial flora of the infant intestine. How does *Lactobacillus bifidus* get its name? What significance is the fact that this organism predominates in the infant intestine?
2. Describe the distribution of bacteria in the adult intestine and name some of the principal kinds usually present.
3. What importance have the bacteria in the normal intestine for the health of the individual? Explain the influence of diet upon the kinds of bacteria in the intestine.

4. What are the principal organisms constituting the colon-typhoid-dysentery group of bacilli? Why are they classed together as one group? What fermentation reaction distinguishes *Escherichia coli* and *E. communior* from other members of the group?
5. Give the facts of outstanding importance concerning the distribution, principal properties and disease-producing activities of each of the following organisms: *Escherichia coli, Proteus vulgaris,* and *Alkaligenes fecalis.*
6. Name three common nonspecific infections of the intestinal tract, and explain how the infection may occur. What organisms are most commonly concerned in each?
7. What is the real nature of the disease called "ptomaine poisoning"? What are the organisms most commonly responsible?
8. What types of foods are most likely to be associated with food poisoning and why? Explain how the food may be contaminated. What should be done to prevent bacterial food poisoning?
9. What is the disease called botulism? What does the name mean? Name and describe the bacterium responsible for this disease.
10. Explain why botulism is always due to the eating of a food which has been preserved in some way. Outline the principal symptoms of botulism. What causes these symptoms? Does botulism occur in animals? What procedures would prevent botulism?

CHAPTER XXXVI

TYPHOID AND PARATYPHOID FEVER. DYSENTERY. CHOLERA

THERE ARE four common specific infections which primarily involve the intestinal tract. These are *typhoid fever, paratyphoid fever, dysentery, and cholera.*

TYPHOID FEVER

In olden times typhoid fever was one of the most prevalent, and the most deadly, of human diseases. But during the past century, and particularly in the last thirty years, there has been a marked reduction in the incidence of this disease. One of the most striking features in the history of public health is the decline of typhoid fever, since 1900, from its former position as one of the principal causes of death to its present relatively insignificant place in the mortality tables. The typhoid death rate of the United States as a whole dropped from 35.9 per 100,000 population in 1900, to 2.4 in 1936. For most of the large cities the rate is still lower.

There are now fewer cases of typhoid fever in proportion to population in large cities than in small towns and rural regions. This is due to the fact that sanitation in such small communities is backward, and in particular the facilities for safe disposal of sewage and for the control of the purity of the milk and water supply are inadequate.

Eberthella typhi. The germ of typhoid fever was first described by Eberth in 1880, and was first isolated and studied in pure culture by Gaffky in 1884. This organism is not naturally pathogenic for animals, and typhoid fever cannot be produced in the usual laboratory animals by the inoculation of the bacilli. It has been possible to cause a disease similar to human typhoid in chimpanzees by feeding them the organisms.

Morphology and staining. Like the other members of the colon-typhoid-dysentery group, the typhoid bacillus is a small Gram-negative nonspore-bearing bacillus. There is nothing to distinguish it in stained smears under the microscope from the normal inhabitants of the intestine (colon bacilli) on the one hand, or from other pathogenic members of the group (paratyphoid and dysentery organisms) on the other. It has no capsule. It is actively motile.

Physiological properties. The typhoid bacillus grows readily on the simplest culture media and at room temperature, as well as at body temperature. Its fermentation reactions help to differentiate it from other intestinal organisms (see table p. 479). The one point that needs to be remembered in this connection is that the typhoid bacillus, in common with the paratyphoid and dysentery bacilli, *does not ferment lactose*, and on this basis it can be differentiated from the colon bacilli always present in feces.

Typhoid bacilli are killed by heat and by chemical disinfectants about as readily as most other nonspore-bearing organisms. If present in milk, they would be destroyed by the process of pasteurization (heating the milk to 60°–65° C.). They are able to survive outside of the body in some circumstances, however, much longer than other germs, and this accounts for the fact that typhoid has often been transmitted through contaminated drinking water, milk or other food. In feces, deposited in a privy vault or on the ground, typhoid bacilli tend to die out rapidly, but a few might still survive after twenty-four hours or more, and if it were wintertime, the germs might remain alive in the cold or frozen fecal mass for many weeks. They are said to survive in sewage-polluted water for about a week. In oysters or other shellfish, they may live for a month. In milk, or milk products, such as ice cream or cheese, they are able not only to survive, but to multiply. Nevertheless, typhoid germs have no natural or continued existence outside of the human body. It merely happens that they have a somewhat greater resistance to environmental conditions than most of the germs causing communicable diseases, and, therefore, are sometimes carried from person to person by such remote means as contaminated water or food.

There is no conclusive evidence to show that *Eberthella typhi* produces an exotoxin. It has been demonstrated, however, that the bodies of typhoid bacilli are in themselves toxic when ground up or otherwise disintegrated, due to the endotoxin thus released.

Typhoid bacilli in the body during the disease. In order to bring about typhoid fever, the typhoid bacillus must reach the intestine through the mouth. Following entrance of the germ, there is always an incubation period of seven to fourteen days, averaging about ten days, before symptoms appear. During this time the organisms penetrate the wall of the upper intestine, causing an inflammation, especially in patches of lymphoid tissue (Peyer's patches) in the intestinal wall. They probably pass into the mesenteric lymph glands very early and thus reach the lymph channels, then the blood stream, and are carried all over the body. They may localize in various internal organs, but especially often in the spleen, bone-marrow, and gall bladder. At the time symptoms begin, there is nearly always a *bacteremia*, that is, the typhoid bacilli are present in the circulating blood. In its early stages typhoid fever is a more or less generalized infection, not confined to the intestinal tract. One evidence of this is the appearance, during the first few days of illness, of the characteristic "rose spots," which are really little colonies of typhoid bacilli in the skin. Serious complications may arise as the result of growth of the germs in other parts of the body than the intestine. In the later stages of the illness, however, the infection is most active in the intestinal tract.

The accompanying chart (Fig. 85), which is based on the findings in many different cases of typhoid fever, averaged together, gives a good idea of the usual course of events during the disease. The typhoid bacilli begin to disappear from the blood during the first week of the illness, and especially rapidly after the second week, so that they can be found in the blood stream only rarely after about the middle of the third week. In individual cases, of course, there may be variations from this general trend. In favorable instances the bacilli may all disappear from the blood within the first few days of the illness, whereas in fatal cases the bacteremia may persist until death.

The disappearance of the organisms from the blood is clearly associated with the development of specific antibodies against the germs. We learn from the chart that agglutinins for typhoid bacilli can be demonstrated in the serum of most patients toward the later part of the first week of illness, and in a still higher proportion of cases later in the disease. These antibodies in any particular case reach their maximum about the time of recovery, then they gradually decrease in amount, but can still be detected in the patient's serum weeks or months later.

Even before symptoms begin, that is, during the last days of the incubation period, typhoid bacilli may be found in the feces, and they are eliminated in these excreta, and in many cases in the urine also, throughout the illness, and often in the days of convalescence as well. They become most abundant in the feces during the second and third week of the disease. This probably means that there is a secondary localization of the infection in the intestine after the germs have largely disappeared from the circulating blood and other places in the body. Sometimes the inflammation in the intestinal wall is so severe, as a result of invasion of the damaged mucous membrane by other bacteria besides the typhoid bacilli, that the wall is actually *perforated*,

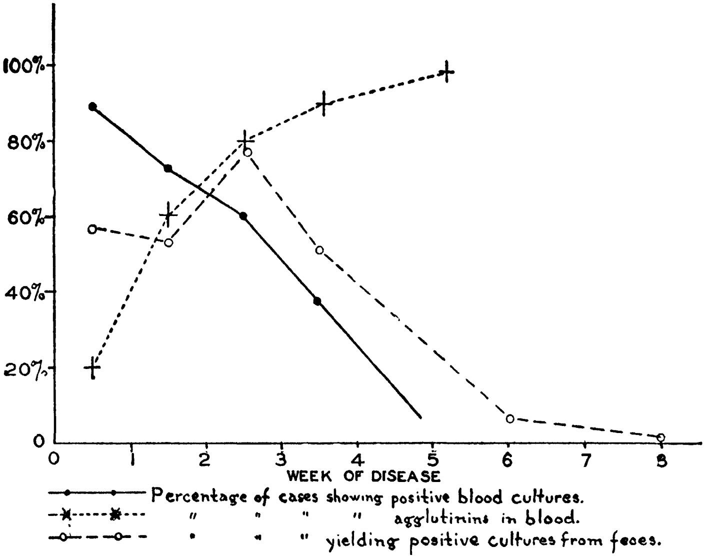

FIG. 85. Chart showing the course of events in typhoid fever. (Redrawn from Topley and Wilson.)

causing a fatal peritonitis. In most cases, however, the infection in the intestine gradually subsides, and the intestinal wall eventually returns to its normal state.

It is most important to realize that recovery from the illness does not mean that all of the germs have disappeared from the excreta. A striking feature of typhoid infection is the tendency of the bacilli to persist in the body, and to appear in the feces or urine, at intervals, for long periods after recovery. In approximately 5 per cent of cases the germs may still be found in the excreta for as long as three months after recovery (such an individual is called a *convalescent carrier*), and in a smaller percentage of cases the recovered patient becomes a *chronic carrier* for years thereafter and perhaps for the rest of his life. In the case of chronic carriers, the germs most often persist in the gall bladder, and reach the intestinal contents through the bile; or they may localize in some part of the urinary tract, and be passed in the urine. Such carriers are often discovered only after they have innocently transmitted the disease to others, and the presence of the germs in their excreta has been demonstrated by laboratory tests.

How typhoid spreads. We have already mentioned the principal ways by which the germs of typhoid fever may be carried from person to person. Whatever the mode of transfer may be, *it is the intestinal discharges or urine of typhoid cases or carriers which are the real sources of typhoid germs*, and new cases arise when these germs reach the mouths of susceptible persons.

The infection may be acquired through personal contact with cases or carriers by way of contaminated fingers. Transfer of typhoid infection in this way is always an important factor in any outbreak of the disease. Vegetables, salads, and similar foods may be the vehicle by which the germs are carried, when they are contaminated by the fingers of the cook. Oysters, and other shellfish, which haye been grown or fattened in sewage-polluted waters, may harbor typhoid bacilli, and infection may follow when these are eaten in the raw state. At the present time the most common source of typhoid outbreaks, especially in the larger cities, is contaminated (raw) *milk*. When milk-borne epidemics are investigated, the trail usually leads to a single dairy and to a single individual milker, or other dairy worker, who has a mild case of typhoid or who is a carrier. Finally, typhoid fever has often been caused by a contaminated public *water* supply. Water-borne epidemics are now uncommon in cities, because of the almost universal use of artificial water purification methods. Small outbreaks in country districts are frequently traced to contaminated well water.

As in many other infectious diseases, mild cases of typhoid fever, not showing the usual signs of the typical disease, are common, and these often go unrecognized, neither the patient nor anyone else being aware that he is infected with typhoid germs. In young children, especially, such cases are often overlooked. It is these mild, unrecognized cases, and the healthy carriers of the germs, which constitute the greatest danger to the public health.

The importance of *carriers* in the spread of typhoid fever can scarcely be overstated. Chronic carriers, who harbor the germs for years, have often been responsible for outbreaks of the disease. A famous case is that of "Typhoid Mary." This woman served as cook for several families in New York State. Within ten years' time, seven outbreaks of typhoid fever occurred which could be clearly traced to her. She refused to cooperate with health authorities and would not give up her occupation as cook, or submit to an operation for removal of the gall bladder, which in all probability would have freed her of the germs. She was forcibly detained in a New York hospital for three years, and finally released on her promise not to engage in cooking. A few years later she turned up again as the cook in an institution where an outbreak of typhoid had occurred. Her complete history has never been learned, but she is known to have been responsible for at least 51 typhoid cases. She spent her declining years as a ward of the State of New York on North Brother Island, and died there in November, 1938.

Bacteriological diagnosis. A laboratory diagnosis of typhoid fever may be made by: (1) demonstrating the presence of *Eberthella typhi* in (*a*) the blood, or (*b*) the feces or urine of the patient, or (2) by demonstrating the presence of specific antibodies (agglutinins) for the bacillus in the patient's blood. This latter test is called the Widal test, after the man who first developed the method. The routine procedures for the diagnosis of a suspected case of typhoid fever include: (1) *blood cultures*, (2) *feces (or urine) cultures*, and (3) *agglutination (Widal) tests.*

Blood cultures are made by inoculating 5 c.c. or more of the *patient's blood* directly into a liquid medium. Often a bile broth is used because typhoid bacilli grow particularly well in such a medium. The blood-broth mixture is then incubated for twenty-four hours. If this culture is made from a typhoid patient during the early course of the disease, it will usually yield a pure growth of *Eberthella typhi.* Identity of the organism in the blood culture is determined by microscopic examination, inoculation into various sugar media for fermentation tests, and finally by agglutination tests in known antityphoid serum.

Feces cultures are made by streaking a portion of the diluted feces on plates of a specially prepared agar medium. This medium may be made up according to any one of several commonly used formulae, and named accordingly, for example, eosin-methylene blue medium, litmus-lactose agar, or Endo's medium. The latter is probably the most widely used. But whatever the name, or the details of its composition, the medium has the same essential ingredients. It is an agar medium containing *lactose* (milk sugar) and an *indicator* of some kind. An indicator is a substance, like litmus, for example, which will change its color as the reaction changes toward the acid or the alkaline side. *Since acid is a product of fermentation, any organism that ferments the lactose in the medium will cause the indicator to change color.*

It will be remembered that *Escherichia coli ferments lactose*, whereas *Eberthella typhi* and other pathogenic bacilli of the intestinal group *do not.* Hence, colonies of the colon bacilli will be conspicuous, because they will cause the indicator to change in color toward the acid side. On Endo's medium, for example, these colonies become a bright red, whereas colonies of bacteria that do not ferment lactose—typhoid, paratyphoid, and dysentery bacilli—are colorless. Also, the colon colonies have a peculiar iridescent sheen which makes their recognition easy. This is of advantage to the bacteriologist, because he wants to *avoid* these organisms and to find the colorless colonies of typhoid bacilli. After the plates have been incubated for twenty-four hours sev-

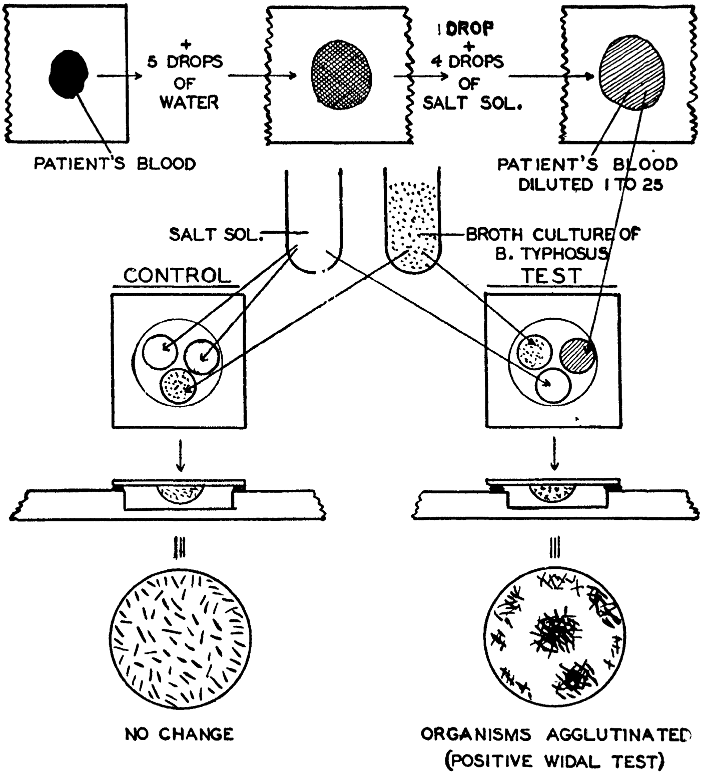

Fig. 86. A method, formerly widely used, for setting up a Widal test (microscopic agglutination test with the patient's blood), and the results when the test is positive. The control must always be set up at the same time and observed under the same conditions as the test drop. In more acceptable methods now generally employed, the test here depicted is repeated with several different dilutions of the patient's blood, accurately prepared from his blood-serum.

eral of the colonies that might contain the typhoid bacilli are carefully "fished" (avoiding *coli* colonies), and inoculated into other media. The pure cultures thus obtained are identified by microscopic examination, fermentation tests, and agglutination tests in known antityphoid serum.

If the case were one of paratyphoid fever or dysentery, the germs of these infections would be isolated from the feces in just the same way. Also, carriers of any of these organisms are detected by the same method of examining the feces. Cultures from the *urine* are made on the same type of media and the germs isolated by the same technique.

Agglutination (Widal) tests are made by mixing a known culture of *Eberthella typhi* with the *patient's serum* and observing the preparation to see if the bacilli are clumped together (agglutinated). Blood for this test may be secured by bleeding the patient from the arm vein and collecting aseptically the serum which separates out when the blood clots. It should be noted that this serum will ordinarily be sterile and in any case we are *not looking for typhoid bacilli* in it, but are going to test it for the presence of specific typhoid *antibodies.* The serum may be mixed with known typhoid bacilli either in a hanging drop (the original Widal technique) or in test tubes.

Proper performance of the test requires attention to numerous technical details. It was formerly the custom to dilute a drop of dried blood (collected from the patient on a clean slide), with salt solution, and to mix this diluted blood with a drop of a living typhoid culture so as to make a final dilution of about 1:75 (Fig. 86). More accurate methods are now generally employed for dilution of the patient's serum (starting with fluid serum) and standardized suspensions of typhoid bacilli of known agglutinability, killed by formalin, are more widely used than living cultures as the known antigen in the tests. Always a series of graded dilutions of the serum to be tested is made from 1:20 through 1:1,280, and each of these dilutions is set up separately with the standardized typhoid suspension in hanging drops or in test tubes. The important point is not the mere presence or absence of agglutinins for typhoid bacilli in the patient's serum, but the concentration of these antibodies, i.e., the *titer* of agglutinins. It is not uncommon for the serum of persons not infected with typhoid germs to agglutinate the bacilli in dilutions of 1:20 or 1:40, but in frank cases of typhoid fever the titer is at least 1:100 and usually considerably higher.

In most diagnostic laboratories, parallel tests to those with typhoid bacilli are set up routinely with the paratyphoid bacilli (*Salmonella paratyphi* and *schottmülleri*) whenever a serum from a patient suspected of having typhoid fever is submitted for examination. In this way cases of paratyphoid fever may be discovered.

Interpretation of laboratory findings. All of these methods of diagnosis—blood cultures, feces or urine cultures, and Widal tests—are tried in any case of suspected typhoid fever, and repeated until a positive diagnosis is made, but it will be clear, from the outline of the usual course of the infection given above, that not all of the methods are equally valuable as diagnostic tests at all stages of the disease. A single negative result with any of these methods does not rule out typhoid infection. Technical difficulties sometimes account for failure to get a positive result. On the other hand, it must be remembered that the finding of *Eberthella typhi* in the feces or urine does not in itself prove that the patient has typhoid fever, for he may be a *carrier*, and a positive Widal test may be given by *immune* as well as infected individuals. It is often necessary to repeat the various tests several times, and they must always be interpreted in the light of the *patient's clinical condition and previous history*, before a diagnosis of typhoid fever can be made with certainty.

Especial caution must be used in interpreting the results of Widal tests. It does not follow that every patient who shows typhoid agglutinins in his blood, is suffering from typhoid fever. Instead, the individual may be *immune*, either as a result of recovery from an attack of typhoid sometime in the past, or because he is a carrier of the organism, or has been vaccinated against the disease. A low titer agglutination (for example 1:40) in the Widal test may be due to the fact that the patient has not yet formed sufficient antibodies, and a test made a few days later may be strongly positive in a much greater dilution. If a change from a low to a higher titer of typhoid agglutinins can be demonstrated in successive samples of blood from the same patient during the course of the disease, this is the most perfect proof that the infection is due to *Eberthella typhi*, because only this organism could cause the formation of antibodies specific for itself.

Immunity. Following recovery from an attack of typhoid fever, an individual has a high and lasting immunity to this infection. The resistance is not absolute, but it is so strong that second attacks of typhoid are rare. The individual is not immune, however, to paratyphoid infections.

A high degree of resistance to both typhoid and paratyphoid infection may be obtained by active immunization with a vaccine consisting of a suspension of killed typhoid and paratyphoid bacilli, as described below.

Serum treatment for typhoid fever is being tried seriously at the present time, but as yet is not beyond the experimental stage.

Prevention. From what has been said about the spread of typhoid fever, it will be clear that prevention of this disease depends in part upon certain public sanitary measures for which municipal and state health authorities are primarily responsible, namely: (1) an efficient sewage disposal system, (2) a pure water supply—in cities this usually requires artificial purification of the water, (3) a pure milk supply, which involves sanitary control of dairies and of the marketing of milk, and pasteurization, (4) sanitary control over the preparation and marketing of foods, especially milk products and shellfish, (5) frequent medical and laboratory examination of all persons who handle foods for public consumption and the exclusion of typhoid carriers from such occupations, and detection and control of other carriers so far as this is possible, and (6) the extermination of flies. These public measures have been remarkably successful and typhoid fever is one of the few diseases which is directly affected by sanitary conditions, and is always greatly reduced in frequency when these conditions improve. But typhoid must continue to spread by personal contact, unless individual cases of the disease are promptly diagnosed and very carefully *isolated.* The responsibility here lies with the physician and the nurse. The nurse, especially, must know how to protect herself, the members of the patient's family, and the entire community from the spread of the disease.

If the patient remains at home, the nurse should avoid intimate contact with other members of the household, and in particular should have nothing to do with the preparation of their food. Flies must be kept out of the sickroom. Concurrent disinfection of the patient's dishes, linens, etc., and all the other necessary procedures in caring for an isolated patient, must be carried out most conscientiously. Especial effort must be made to disinfect promptly and thoroughly all of the feces and urine. The best disinfectants for this purpose are chlorinated lime, cresol, or carbolic acid. For the feces, the disinfectant must be used in strong concentration and in at least twice the volume of the mass to be disinfected. It must be mixed thoroughly with all parts of the material and must be allowed to act for at least one hour. It is important to remember that in intestinal infections the greatest danger lies in the possibility of conveying the germs to the mouth through the contaminated hands. It is, therefore, essential that the nurse and other attendants disinfect their hands immediately after every contact with the patient. These precautions must be continued not only throughout the illness, but into the days of convalescence as well, *until repeated laboratory examinations show that the patient is no longer excreting typhoid germs.*

Prophylactic vaccination. Finally, a most important measure for the prevention of typhoid fever is *prophylactic vaccination.* The vaccine consists of a suspension in buffered salt solution of *Eberthella typhi* killed by heating for an hour at about 56° C. and preserved by the addition of 0.25 per cent tricresol or 0.5 per cent phenol. A triple vaccine, containing paratyphoid A and B bacilli as well as the typhoid bacilli, was formerly widely used, but is now less commonly employed. Both the U. S. Army and Navy now use a vaccine containing typhoid organisms only. For many years nearly all the typhoid vaccine made in this country was prepared from a single typhoid strain (the "Rawlings strain"). Following a series of investigations which indicated that this strain had changed and was less efficient as an immunizing antigen than more recently isolated and more virulent cultures, the U. S. Army in 1936 began preparation of their vaccine with a new strain (No. 58). Their vaccine contains one billion killed typhoid bacilli per cubic centimeter.

Usually three injections of typhoid vaccine are made subcutaneously at intervals of a week. The effect of the original immunization probably persists for at least three years and may be prolonged by a subsequent *intradermal* injection of 0.1 c.c. of the vaccine. This vaccination is recommended only for immunization of persons exposed to unusual risks of infection, such as nurses, physicians, soldiers and sailors, campers and travelers.

It must be remembered that, despite the apparent effectiveness of prophylactic vaccination, the vaccinated individual may still contract the disease if he is exposed to a particularly heavy dose of virulent germs, and, whether vaccinated or not, the same care must be exercised to avoid infection.

PARATYPHOID FEVER

This infection is in every way similar to typhoid fever. In general, however, it tends to be less severe. The causative organism is *Salmonella paratyphi,* or a closely related member of the paratyphoid group. The mode of infection, methods of laboratory diagnosis, and prevention, are essentially the same as in typhoid.

The numerous varieties of the paratyphoid bacilli, as we have previously pointed out, are commonly associated with acute bacterial food poisoning in man. They are also responsible for naturally occurring intestinal infections in many kinds of animals.

DYSENTERY

The term *dysentery* is really a clinical expression meaning simply an illness characterized by diarrhea. One type of dysentery is due to an ameba—*Endameba histolytica*—and is called *amebic dysentery.* This disease is discussed in Chapter XLI. The type of dysentery with which we are concerned here is *bacillary dysentery,* caused by members of a group of bacteria belonging to the same family as the germ of typhoid—the dysentery bacilli.

Varieties of dysentery bacilli. There are two main varieties of these organisms, *Shigella dysenteriae,* first isolated by Shiga in 1898 during an epidemic of dysentery in Japan, and *Shigella paradysenteriae,* of which the *Flexner* subtype, first obtained by Flexner in 1900 from cases of the disease in the Philippine Islands, is the most important. The Shiga bacilli form a distinct species, and are set apart from the other types by their capacity to form a powerful *exotoxin.* Under the name *Shigella paradysenteriae* there are usually included the varieties called Hiss (Y), and Strong, as well as the Flexner type.

Special mention must be made of a peculiar variety of dysentery bacilli, first described by Duval, but now generally referred to as the Sonne type (no species name is used). These organisms differ from all the pathogenic intestinal bacilli we have been describing in the fact that they cause a slow but definite fermentation of *lactose.*

Outbreaks of dysentery due to the Shiga bacillus occur most frequently in Asiatic countries. In the United States the Flexner and Sonne types of dysentery bacilli are responsible for the great majority of cases.

All of these dysentery bacilli, as well as other varieties and subvarieties we have not specifically mentioned, are Gram-negative, nonspore-bearing bacilli, like typhoid and colon bacilli, but distinguished from the latter by one conspicuous property —they are *not motile.*

The disease in man; diagnosis. Like typhoid, dysentery is a human disease, and the germs do not naturally infect animals. *Acute* dysentery differs from typhoid in having a short incubation period of rarely more than forty-eight hours, and in being strictly an intestinal infection. In dysentery, the germs are not found in the patient's blood and they do not invade the internal organs to any great extent. They cause a severe inflammation of the walls of the intestine, often resulting in permanent damage. *Chronic* dysentery, assuming many clinical forms, is common, especially in the southern United States and other warm regions.

The only satisfactory method of laboratory diagnosis is to cultivate the bacilli from the stools. In the early stages of acute dysentery this is accomplished without difficulty by using the same technique as that employed for isolation of typhoid bacilli. In milder cases, chronic cases or carriers, however, recovery of the germs from the feces is difficult and uncertain. Agglutinins can usually be demonstrated in the blood of patients a week or two after an acute attack and these antibodies may be present in cases of chronic infection also. A titer of 1:160 is commonly regarded as significant. Results of all such tests with patients' serum must be interpreted with great caution, however, and their true meaning can be judged only in the light of the full medical history of the individuals concerned.

The spread of dysentery; prevention. Dysentery is typically an epidemic disease. It is especially liable to occur among soldiers in the field, and among the inmates of prisons, asylums, and other institutions, where men are crowded together under poor hygienic conditions. Carriers of one or the other variety of dysentery bacilli are often responsible for outbreaks of the disease. The infection spreads largely by personal contact, and through the agency of flies, and also may be transmitted through contaminated food or water.

Like other intestinal diseases, dysentery is largely prevented by good sanitary facilities. The dysentery organisms contain so much endotoxin that they cannot be injected safely in the form of a vaccine into human beings, unless their toxicity is in some way reduced, as by mixture with antiserum or treatment with formalin, and active immunization against the disease is not often attempted. Dysentery is still a serious problem in armies at war and is likely to become epidemic in any circumstances where sanitation is inadequate.

Summer diarrhea. An important form of dysentery is the so-called *summer diarrhea*, or *infantile diarrhea*, which becomes prevalent everywhere in the hot months of the year. A large proportion of the deaths of infants under two years of age are due to this disease. Breast-fed babies usually escape the infection, but it is frequent among bottle-fed infants, particularly in homes where there is poverty, overcrowding, uncleanliness, and generally poor hygienic conditions. When the feces of infants suffering from diarrhea are examined, bacilli of the same varieties as those associated with dysentery in adults are sometimes found, but in many instances organisms of other kinds seem to be responsible. Among the bacteria most frequently encountered are *Proteus vulgaris* and *Clostridium welchii.* Summer diarrhea in bottle-fed infants can be prevented only by extreme care in the preparation of their food. All water and milk they receive should be boiled, and the bottles and nipples should be sterilized. If an infant can be breast-fed during its first summer, most of the danger will be avoided.

CHOLERA

Cholera is caused by infection of the intestine with a *spirillum*, discovered by Koch in 1884, and named *Vibrio cholerae.* The disease is marked by a severe diarrhea with watery stools, cramps, prostration, and suppression of urine. In many outbreaks, a large proportion of patients die, though mild cases also occur. The real home of cholera is in India, particularly along the delta of the Ganges River. From this focus the disease has many times swept in severe epidemics over most of the world, and numerous cases appeared in America. But since about 1875, cholera has been kept out of the United States and England, and with the development of Western civilization, it has been gradually restricted to those countries where modern sanitation is as yet lacking. In a few places, such as Persia, Arabia, parts of China, and Russia it is still a great scourge.

There is little likelihood that cholera will ever again get a foothold in this country. It is necessary, however, for authorities of the United States Public Health Service to maintain a constant watch at ocean ports to prevent the admission into the country of immigrants who are carriers of the spirilla. To this vigilance we owe our present freedom from the disease.

Vibrio cholerae. Cholera spirilla appear most typically as short spirals in the shape of a comma; for this reason the germ is often referred to as the "comma bacillus." In smears from "nests" of the organisms in the feces, they appear lined up with each "comma" headed in the same direction like fish in a stream. In cultures, longer and more irregular, curved forms are seen. The spirilla are actively motile, and Gram-negative. They can be cultivated in the laboratory upon ordinary media. They liquefy gelatin and coagulated blood serum. In broth they form indol and they also reduce nitrates to nitrites. When a broth culture is made strongly acid by the addition of hydrochloric or other mineral acid, a deep red color appears, due to the presence of nitroso-indol. This is the so-called "cholera-red test." The organisms do not produce an exotoxin, but they contain considerable endotoxin.

Laboratory diagnosis. Infection with the spirilla occurs when the germs enter the mouth by way of contaminated fingers, food, or water. The germs remain confined to the intestine and appear in large numbers in flakes of mucus in the watery feces. A provisional diagnosis may sometimes be made by a simple microscopical examination of these mucous flakes, but in doubtful cases a definite diagnosis can be arrived at only after the spirilla have been isolated from the feces and carefully identified. Carriers are detected by feces examination and tests for cholera agglutinins in the blood.

The spread of cholera; prevention. Cholera spreads by the same means as other intestinal infections discussed above. Waterborne epidemics have been especially common. Measures to prevent cholera are similar to those for typhoid. In most countries, stringent regulations are enforced by health authorities at seaports to prevent the introduction of the disease by immigrants from places where cholera is endemic. Recovery from cholera confers a lasting immunity. A vaccine, consisting of a suspension of the dead or attenuated cholera spirilla, has been tried for prophylactic immunization. Castellani has combined this with the triple typhoid vaccine. The evidence indicates that cholera vaccination has considerable value.

REVIEW QUESTIONS—CHAPTER XXXVI

1. Name the four common specific infections of the intestinal tract.
2. Discuss the prevalence of typhoid fever. What are some of the factors which have caused it to be less common?
3. Name and describe the morphological and physiological properties of the germ of typhoid fever.
4. Trace the typhoid bacilli through the body during the course of typhoid fever. When are the germs to be found in the blood? In the feces? When do specific antibodies appear in the blood?
5. Explain how the germs of typhoid may be carried from person to person. What is the importance of personal contact? Of carriers?
6. In what two ways may a laboratory diagnosis of typhoid fever be made? What are the three routine diagnostic procedures?
7. How is a diagnosis made by blood cultures? At what time during the infection are they most likely to reveal the presence of *Eberthella typhi?*
8. Outline the method of examining feces for typhoid bacilli. How must the results of feces cultures be interpreted?
9. Explain in detail the method of performing a Widal test. In this test what are you looking for? In a positive test what is agglutinated? What causes this agglutination? Discuss interpretation of the results.
10. How may immunity to typhoid infection be acquired?
11. Discuss fully the prevention of typhoid fever. Explain the importance of (*a*) sanitation and the work of public health authorities, (*b*) care of the isolated case, and (*c*) prophylactic vaccination. Describe the present U. S. Army vaccine.
12. What is paratyphoid fever? What is the importance of paratyphoid organisms in human disease?
13. What is "dysentery"? What type of dysentery is caused by a protozoan? Name and describe the principal varieties of the dysentery bacilli.
14. Outline the nature of the infection, laboratory diagnosis, mode of spread, and prevention of bacillary dysentery.
15. Discuss the nature, cause, and prevention of summer diarrhea.
16. Where does cholera occur? How is it kept out of the United States?
17. Name and describe the principal properties of the germ of cholera. Discuss cholera briefly as to nature of the disease, laboratory diagnosis, mode of spread, and prevention.

CHAPTER XXXVII

TUBERCULOSIS

TUBERCULOSIS, in various clinical forms, has been for centuries one of the most common and most fatal of human diseases. It attacks persons of all ages and all races the world over. Among the crowded populations of cities it has been a veritable plague. At the present time its wide prevalence, chronic nature, with the consequent great loss of time and money on the part of its victims, and its close relationship to general health conditions within a community, combine to make tuberculosis a disease of outstanding importance and of more than ordinary significance for the well-being of the people as a whole. It attacks individuals in so many families, and the measures which help to prevent it are so fundamental for the promotion of health in general, that efforts to control it have a very prominent place in the public health program of every community.

In olden times tuberculosis was not only exceedingly common, but it was also a severe, acute disease, often rapidly fatal. It still may have this character when it occurs in infants, or in individuals of a savage race not often exposed to the infection. But for the past hundred years or more the disease has steadily decreased in severity in all of the more highly civilized countries of the world. This change may be due, in part, to a gradual rise in the average level of resistance to tuberculosis among the members of the population, but probably the general sanitary and hygienic reform which has taken place during this time has had the greatest influence. The decline has been especially marked during the past forty years. In 1900 the death rate from tuberculosis per 100,000 population in the United States was 201.9, while in 1936 it was only 55.7. *Nevertheless, tuberculosis is still the most prevalent of all germ diseases* (except, perhaps, gonorrhea), and although it tends now to be chronic, and less severe than formerly, it is still one of the major causes of death. Among the infectious diseases, only pneumonia is now responsible for more fatalities.

Mycobacterium tuberculosis. *Varieties of tubercle bacilli.* Tuberculosis is a common disease in various species of animals as well as in man. The organisms responsible for these infections are not all exactly alike, however. Four distinct types of tubercle bacilli are recognized: (1) the *human,* (2) the *bovine* (cattle), (3) the *avian* (bird), and (4) the *cold-blooded* type. The latter causes a tuberculous disease in frogs, turtles, fish and other cold-blooded animals.

It was Robert Koch, in 1882, who discovered the human and bovine tubercle bacilli, and proved that they cause tuberculosis, but the recognition of the bovine type as distinct from the human was due to other investigators. These two types, human and bovine, are the only ones which may infect human beings.

Morphology and staining. One of the most outstanding characteristics of the tubercle bacilli is their capacity to take an *acid-fast stain.* They belong to the group of *acid-fast bacilli*—organisms that are somewhat difficult to stain, but, when once stained, are not decolorized by acids. Among other members of the group are the *leprosy bacillus,* found in the lesions of leprosy, the *smegma bacillus,* a normal inhabitant of the human skin in the genital region, and certain harmless saprophytic acid-fast bacilli, including the *butter bacillus* and *timothy-grass bacillus.*

The most widely used method of demonstrating acid-fast staining is the Ziehl-Neelsen method described on page 176. The method consists in staining the bacteria with a strong carbol fuchsin solution with the aid of heat, then dipping the slide in acid-alcohol (containing 3 per cent of concentrated hydrochloric acid). This takes out the color from everything *except* acid-fast organisms. Then a counter stain (methylene blue) is applied. With this method, tubercle bacilli appear as bright red rods in a pale blue field (Fig. 87).

The peculiar staining properties of the acid-fast organisms are due to the presence of fatty materials, and compounds in the nature of complex alcohols, in the bacterial cells.

The tubercle bacilli are rod-shaped organisms, of variable length, often slightly curved. They are nonmotile and do not form spores. In cultures they are usually very short rods, but in some conditions long filaments may form. Rods with swollen, clubbed ends, resembling diphtheria bacilli, are sometimes seen.

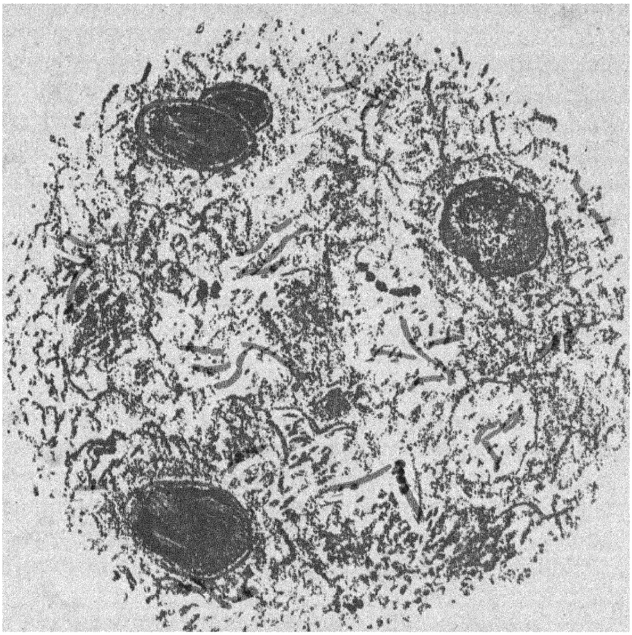

FIG. 87. *Mycobacterium tuberculosis* in a smear of sputum stained by the Ziehl Neelsen acid-fast staining method.

In smears of tuberculous sputum, the organisms are often rather long and slender, and they frequently appear in small groups or bundles with the individual bacilli lying at acute angles to each other. They stain sometimes uniformly, but often unevenly, so that they have a beaded appearance. There are no constant differences in morphology or staining among the four types of tubercle bacilli, and it is impossible to distinguish between them by microscopic observation alone.

Physiological properties of human tubercle bacilli; isolation and cultivation. Mycobacterium tuberculosis is difficult to isolate in pure culture, because it grows much more slowly than other bacteria and does not adapt itself readily to artificial conditions. Isolation from sputum is particularly difficult, but if the sputum is first treated with *antiformin* (a solution of sodium carbonate and chlorinated lime), which will kill most of the bacteria likely to be present *except* the tubercle bacilli, then centrifugalized, and the sediment is inoculated upon a solid medium, such as Petroff's gentian violet egg medium, an almost pure growth of the tubercle bacilli may often be obtained.

The most certain method of isolation, however, is to inoculate the original material into a guinea pig. The animal will develop tuberculosis and in the characteristic tubercles the germs will be found almost free of contaminating organisms. If now, a portion of this tuberculous tissue is carefully removed from the animal with aseptic precautions and rubbed over the surface of a slanted medium, a pure culture of the tubercle bacilli will usually be secured.

Cultural characteristics. Once isolated, the organisms grow moderately well. They multiply so slowly that cultures are usually incubated for one to three *weeks* or more, instead of a day or two, as in the case of the ordinary bacteria. The character of their growth is peculiar and quite different from that of other germs. On a slant of coagulated egg medium or glycerin agar they form irregular, whitish masses, resembling bread crumbs, raised above the surface, very tenacious, and adhering closely to the medium; or in other cases the growth may appear as a dull, dry, wrinkled film. It is hard to prepare a satisfactory smear from such a culture, and the organisms will not emulsify readily in water as most bacteria do, but they settle out of suspension as granular masses. Growths on coagulated serum or egg media eventually develop a yellowish, orange, or brown color. In glycerin broth the human tubercle bacilli characteristically grow as a thick, wrinkled film, covering the surface of the liquid. Cultures have a sweet, fruity odor. These peculiar cultural characteristics, taken in connection with other properties of the organisms, suggest a relationship between the tubercle bacilli and the molds and actinomyces, and have led bacteriologists to classify them with the *higher bacteria* (*Mycobacteriaceae*). (See pp. 46 and 78.)

The tubercle bacilli do not produce exotoxins, but poisonous substances, probably of the nature of endotoxins, are liberated when the bodies of the bacilli are disintegrated. These poisons are doubtless responsible, in part, for the fever, weakness, and other characteristic symptoms of tuberculosis.

Resistance. In common with other acid-fast bacilli, the tubercle bacillus is unusually resistant to chemical germicides, and to drying, but it is as susceptible as other nonspore-bearing bacteria to heat. Several hours are required to kill tubercle bacilli in sputum with a 5 per cent carbolic acid solution. We have already mentioned their resistance to antiformin. In dried sputum or dust, in the dark, they may remain alive for months. On the other hand, they are destroyed by an exposure of twenty to thirty minutes to a temperature of 60° C. Pasteurized milk, then, is free of living tuberculosis germs. Direct sunlight and ultraviolet light destroy them quickly, so that sputum which has been exposed to the sun for an hour or two is not likely to be dangerous.

Differentiation of the human type of tubercle bacilli from the other types and from saprophytic acid-fast bacilli. Among the harmless acid-fast organisms, the smegma bacillus (*Mycobacterium smegmatis*), is the only one likely to be confused with *Mycobacterium tuberculosis.* It sometimes gets into specimens of urine or feces which are to be examined for tubercle bacilli. If an acid-fast organism is found in such a specimen the question of its identity can be settled by the inoculation of a guinea pig. Tuberculosis germs will bring about a tuberculous infection in the animal, but the smegma bacillus is nonpathogenic. All the *saprophytic* acid-fast bacilli develop much more rapidly and profusely on culture media than tubercle bacilli, though in the case of some strains the appearance of the growth is very similar.

They all grow at room temperature (25° C.), whereas none of the tubercle bacilli will grow at temperatures below 30° C., except the cold-blooded type.

The *avian* and *cold-blooded* types of tubercle bacilli are readily distinguished from the human and bovine types by their cultural characteristics and special disease-producing powers. Both develop a luxuriant creamy growth within a few days on slanted media. Neither type is able to cause a progressive infection in guinea pigs; the avian produces tuberculous infection in fowl and pigeons, and the cold-blooded type infects only cold-blooded animals, such as frogs, lizards, turtles, and fish.

The *bovine* type closely resembles the human tubercle bacillus and its differentiation from the human type is sometimes difficult. In cultures, human and bovine strains are very similar. The bovine type, however, usually yields a consistently poorer growth on media of all kinds and its development is not aided by the addition of glycerin to the medium as is the human type. Further, bovine strains do not form pigment as human type cultures do. The most important difference, however, is in the degree of disease-producing power of the respective types for *rabbits.* Whereas both the human and bovine types are very pathogenic for guinea pigs, only the bovine type will produce a generalized, fatal infection in rabbits.

Characteristic features of tuberculous infection in man. Almost any part of the body may be invaded by tubercle bacilli. In children, the infection is often localized principally in the lymph glands of the abdomen or neck or in the bones and joints. Occasionally, the skin is the site of infection (lupus). By far the most frequent form of the illness, however, especially in adults, is a chronic *pulmonary tuberculosis*, in which the lungs are the seat of the infection. In infants, and more rarely in adults, an acute, fatal, generalized infection may occur, known as *miliary tuberculosis,* in which tiny tubercles about the size of millet seeds (hence called *miliary* tubercles) are found scattered throughout the organs of the body. Another fatal form of tuberculosis in children is *tuberculous meningitis.*

Tubercles are fundamentally alike wherever they are found. They form whenever tuberculous infection is present in any solid organ of the body. They begin as microscopic collections of epithelioid cells, arranged about a group of tubercle bacilli. Later, lymphocytes accumulate in this area. The microscopic tubercles enlarge and coalesce with neighboring tubercles. Near their center, characteristic giant cells appear, and in these cells the tuberculosis germs are usually found. Often tubercles become so large that they are easily visible with the naked eye. They appear as tiny gray masses, about the size of a pinhead, and hard to the touch.

In the lungs, or other organs, when the disease is active, many tubercles appear and overlap one another, destroying the normal tissues. The cells in the center of these tubercles die and disintegrate into a soft, cheesy mass, and later, when this matter is cast out of the body, there is left behind an actual cavity in which all the normal tissue has been consumed. In this manner almost the whole of a lobe of a lung may be destroyed, or another organ, such as a kidney, similarly may be reduced to little more than a shell. "Consumption" is an accurate term with which to describe a severe tuberculous infection (Fig. 88).

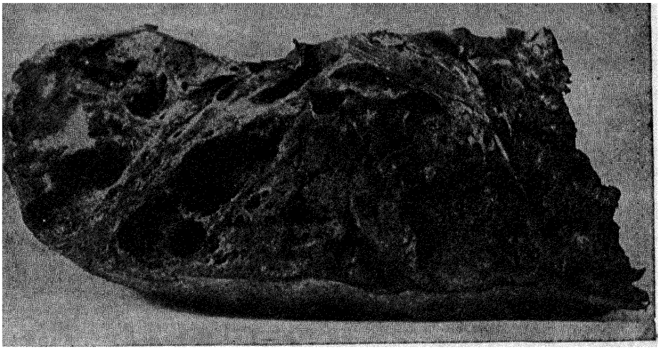

FIG. 88. Portion of the lung removed at autopsy from a case of chronic pulmonary tuberculosis. The specimen has been cut through to expose the large cavities in which the normal lung tissue has been entirely consumed. There is no normal tissue remaining anywhere, the entire lobe is involved in an advanced tuberculous process.

It must not be imagined, however, that tubercle formation and the consequent destruction of the tissues goes on unimpeded. On the contrary, there is a characteristic cellular reaction about the tubercles which eventually results in the formation of a thick envelope of fibrous tissue around them. This seems to constitute the principal defense of the body against the germs; it is an attempt to wall off the infected area and to prevent the spread of the infection further into the healthy tissues. When this defensive process is fully successful the complete healing of the tubercles follows, that is, all the germs present die, and the danger of future spread of the infection from that area is over. Often calcium salts are later deposited in these healed tubercles.

In many instances, however, the body reaction is not sufficient to bring about complete elimination of the germs, but instead the progress of the infection is simply *arrested.* The tubercle bacilli do not all die, but on the contrary, some remain alive in incompletely healed tubercles for long periods, perhaps for the whole of the individual's life. The infection may never advance any further, but there is always the possibility that the germs may start into renewed activity at some future time, when for some reason the resistance of the body is reduced and the cellular defense gives way. The fact that infection with tubercle bacilli may thus remain *latent,* causing no illness, but may later develop into an actively progressing infection with accompanying symptoms of clinical tuberculosis, when resistance is lowered, is a very important and characteristic feature of this disease.

Prevalence of latent infection. Tuberculous infection and tuberculous disease. A point of fundamental significance in the modern conception of tuberculosis is the knowledge that *almost every individual,* at some time, usually in childhood, *becomes infected with tubercle bacilli,* though only a comparatively few persons ever develop signs or symptoms of illness which we recognize as tuberculous *disease.* The majority of individuals have sufficient defensive powers so that the tuberculous infection, which they unconsciously suffer in early life, is confined to a small area only in the lymph glands or lungs, and heals spontaneously, or at least is held in check so well that it never advances far enough to produce recognizable symptoms of tuberculosis.

Two kinds of evidence have made the almost universal prevalence of tuberculous infection abundantly clear. Pathologists who have examined the lymph glands and lungs at autopsy of many hundreds of persons dying of diseases other than tuberculosis, have found healed or partly healed tubercles in a high proportion of individuals. A second kind of evidence is afforded by the results of *tuberculin tests.* Tuberculin is a preparation made from cultures of tubercle bacilli, as described below. Persons who have been infected at any time with tubercle bacilli, or are at the moment infected, will develop a characteristic inflammatory reaction when a small amount of tuberculin is applied to the skin, whereas individuals who have never been infected will give no reaction. It has been found that almost 100 per cent of adults give a positive tuberculin reaction. The proportion of children who react positively increases steadily with age until at about the age of eighteen more than 90 per cent show this definite evidence of tuberculous infection.

There is reason to believe that childhood infection is not now so universal as it once was, but nevertheless, it is still true that a high proportion of adults have a focus of infection with *Mycobacterium tuberculosis* somewhere in the body, and that infection of most individuals occurs during childhood. But since by no means everyone develops the *disease* tuberculosis, it must be that the childhood infection cures itself or becomes latent in most persons, and it is clear that the human body must be possessed on the whole with a very considerable degree of resistance to the tubercle bacilli.

Factors which influence the development of active tuberculosis. The conditions which bring about an advancing tuberculous process and illness in some individuals, while the infection remains latent in others, and the influences which convert a latent to an active infection, are by no means entirely understood. Many believe that when tuberculosis develops in an adult, it is due to the lighting up of the focus of infection acquired in childhood, and that the determining factor is a loss of the general body resistance which had previously held the infection in check. All the evidence does not support this view, however, and it may be that, in some instances, at least, a new infection, with perhaps more virulent organisms, is superimposed upon the original childhood infection.

At any rate, the general body resistance of the individual is a factor of unusual importance in connection with tuberculosis. Resistance seems to depend more upon general bodily vigor, and the capacity to perfect the local defenses against the spread of the germs in the tissues, than it does upon the action of specific antibodies as in other infections. Active tuberculosis is most likely to occur in persons whose normal resistance has been broken down by the weakening effect of other diseases, by malnutrition, alcoholism, too frequent childbearing, or fatigue and overstrain of one kind of another. It may be that some individuals inherit a constitution which makes them somewhat more susceptible to tuberculosis than other persons. But the disease itself is never directly inherited. Tuberculosis runs in families principally because the children in each generation can scarcely avoid receiving a heavy dose of the germs when they are obliged to live in close contact with an adult member of the family who has an active case.

The occurrence of active tuberculosis is influenced by the general conditions under which people live and work. Dusty trades, for example, particularly those in which the worker inhales dust from metals or stone, predispose to tuberculosis. The disease is most frequent among the poorest families, where unhygienic habits combine with overcrowding, overwork, poor nutrition, alcoholism, and frequent attacks of other communicable diseases, to create a very favorable soil for the tuberculosis germs. On the other hand, tuberculosis is much less likely to occur among those who can avoid an exposure to a heavy dose of the germs in childhood, and who are able to live hygienically—that is, persons who from childhood can keep clean, well nourished, and well rested, can avoid serious weakening diseases, and can enjoy the blessings of sunshine, fresh air, wholesome recreation, and congenial work.

Sources and modes of infection. *Spread of the human type.* The *sputum* of patients with active pulmonary tuberculosis—the commonest form of the disease—is obviously a source of great danger, because it is certain to contain great numbers of virulent tubercle bacilli of the human type. The organisms may also be present in other discharges from tuberculous lesions anywhere in the body, and they may be found in the feces. Although these organisms are somewhat more resistant to external conditions, particularly drying, than other germs, they have no natural existence outside of the body, and the human tubercle germs are always passed from one individual to another through some form of personal contact, most often by way of fingers soiled with moist sputum, or by droplet infection. The latter method of germ transfer probably plays an important part in the spread of the germs of pulmonary tuberculosis.

Bovine tuberculosis. Infection of human beings with the *bovine type* of tubercle bacilli is confined almost entirely to children, and particularly to those under 5 years of age. In all cases, infection with these organisms is contracted by drinking *raw* milk from tuberculous cattle. The germs are usually contained in the feces of the infected cows and get into the milk from the dirty flanks or udders of the animals. In children these organisms are able to penetrate the mucous membranes of the throat and nasopharynx (especially the tonsillar tissue), and so reach the cervical lymph glands, or they may pass through the intestinal mucosa and invade the mesenteric glands. They are apparently able to pass through the intestinal wall without causing any lesions in the wall itself. Once having reached the lymph glands, the organisms may be carried by the lymph or blood streams to other parts of the body. Bovine organisms are sometimes responsible for miliary tuberculosis or meningitis in infants. They rarely give rise to pulmonary tuberculosis.

In the United States, bovine infection is not common at the present time, due largely to the remarkable success of the efforts of public health authorities in recent years to eliminate tuberculous cattle from dairy herds.

In order to diagnose tuberculous infection in cattle, the animals are inoculated with *tuberculin.* Infected cows give a local or general reaction (evidenced by fever or other signs), whereas normal cows are not affected by injection of this preparation. Routine tuberculin-testing of the herd at intervals is practiced in all modern dairies, so that any tuberculous animals may be found and eliminated. There is a demand everywhere that all milk should come from tuberculin-tested cows, and there is good prospect for the almost complete disappearance of milk-borne tuberculosis in this country in the near future.

Bacteriological diagnosis. In order to make a laboratory diagnosis of tuberculosis, it is necessary to demonstrate the presence of tubercle bacilli in the sputum (in cases of suspected pulmonary tuberculosis), or in the urine, joint fluid, spinal fluid or other material from suspicious lesions. This may be done either by (1) *microscopic examination,* using the acid-fast staining method, (2) *cultivation of the bacillus,* or (3) *inoculation of the material into a guinea pig.*

To examine sputum for tubercle bacilli, a cheesy or bloody portion of the sputum is selected and spread in a large and rather thick film over a slide. When the smear is dry, it is fixed, then stained by the Ziehl-Neelsen, or some other acid-fast staining method, and searched for acid-fast bacilli. These may be very numerous in advanced cases or very few in early cases. When once the typical acid-fast rods have been found, a diagnosis of tuberculosis is certain. This is one of the very rare instances in bacteriology when a germ can be recognized by microscopic examination alone.

In cases of suspected tuberculosis of the kidney, or other part of the genitourinary tract, specimens of urine are obtained by catheter, centrifugalized, and smears are made from the sediment. Feces are sometimes examined microscopically for tubercle bacilli. In these examinations the possibility of confusion of the smegma bacillus with the tubercle bacillus must be remembered.

It frequently happens that microscopic methods fail to demonstrate the presence of tubercle bacilli in discharges from suspected tuberculous lesions, especially in specimens of urine, spinal fluid, joint fluid, and the like. In these cases the material may be cultured on special media in an attempt to isolate the tubercle bacilli, and also it may be inoculated into a guinea pig.

This animal inoculation must always be done when there is serious possibility of tuberculosis and whenever there is a question as to the identity of acid-fast bacilli that have been found. The guinea pig is so susceptible that it will develop tuberculosis even though the living tubercle bacilli in the injected material are very few indeed—so few that they cannot be found by any method of microscopic examination. Hence, this is a most delicate test for the presence of tubercle bacilli in any material. Four or five weeks after the injection, the animal is killed and examined for evidences of tuberculosis. If these evidences are found, this is proof that the original material from the patient contained tubercle bacilli.

Immunity. The tuberculin reaction. We have already explained that resistance to the original infection with tubercle bacilli seems to depend principally upon the general bodily health, and particularly upon the capacity to wall off the tubercles and so prevent their advance, rather than upon the action of specific antibodies. Antibodies are formed, nevertheless, and can be detected by proper tests.

With respect to susceptibility to a *new* infection, the situation seems to be the same as in syphilis. So long as there are living tubercle bacilli in the body, though the infection may not be advancing at all (*i.e.*, latent), the individual is *less* susceptible (though not entirely immune) to a fresh infection than a person who has never been infected. If the original tuberculous infection is completely cured, the individual probably becomes, after a time, as susceptible to a new infection as he was in the first place. Since a large proportion of individuals have had a limited tuberculous infection by the time they have reached their eighteenth year, and since this infection probably becomes latent, rather than entirely healed in most cases, it must be true that a majority of adults are relatively resistant to a new infection.

Koch was the first to note that an animal already infected with tuberculosis is more resistant to a second injection of the germs than a normal animal. On the second injection of the bacilli into a tuberculous guinea pig, there is a prompt and severe reaction which is due to the fact that the animal has become *hypersensitive* to the tubercle bacillus or its products. The inoculated organisms are destroyed in the course of this reaction and do not set up a fresh infection. A tuberculous animal is, then, at once more sensitive and more resistant to tubercle bacilli than a normal animal. The situation is the same in human beings.

Koch's Old Tuberculin. Koch thought that by inoculating persons deliberately with a preparation containing dead, disintegrated tubercle bacilli and their products, he might be able to further increase the resistance of tuberculous individuals and bring about a cure. With this in mind he prepared *tuberculin.* He grew tubercle bacilli for six weeks in flasks of 5 per cent glycerin broth, then evaporated the broth to 1/10th its volume by heating it in a water-bath at 100° C. (this heating, of course, killed the bacilli), and passed the concentrated broth through a bacteriological filter. The filtrate was a clear, brown liquid, free of living germs, but containing all the disintegration products of the tubercle bacilli, any substances formed from the medium by the growing organisms, and the concentrated medium itself. This is the preparation now generally known as *Koch's Old or Original Tuberculin* ("O.T.").

Koch found, as he expected, that tuberculous individuals give a reaction when inoculated with tuberculin, whereas normal individuals do not. On his suggestion, physicians attempted to treat cases of tuberculosis by injections of tuberculin. The results were tragic. For a person infected with tubercle bacilli is so *hypersensitive* to tuberculin that very severe reactions follow, and even sudden death, when the dose is too large. The reaction to tuberculin is *local* (about the site of the injection), *focal* (in the lungs or other infected areas), and *general* (evidenced by fever and other symptoms). When the dose is sufficiently small, however, only a local reaction occurs and the individual is not harmed in any way. The use of tuberculin for treatment is, then, a rather dangerous procedure and is now practically abandoned, except for certain selected types of cases and in the hands of experts.

Purified protein derivative (P.P.D.); diagnostic tuberculin reactions. Koch and many others following him have prepared different forms of tuberculin, but only recently has a preparation been available that is definitely superior to O.T. The new tuberculin is a refined, concentrated product representing the active principle of the old tuberculin in almost a pure form. Strains of *Mycobacterium tuberculosis* are grown on synthetic medium and the tuberculin is precipitated out of the heated filtrate by trichloracetic acid. It is then washed and concentrated by ultrafiltration. The purified protein derivative thus obtained is known as "Tuberculin P.P.D." It is put up in tablet form in two strengths, and is now having a wider use than any other form of tuberculin.

Since only infected individuals react, the use of tuberculin preparations for *diagnosis* has naturally been tried. Among the various methods for making tuberculin tests for this purpose, the one formerly used most commonly for human beings is the *cutaneous test of von Pirquet.* The forearm is cleansed with alcohol and ether and two small scratches are made a few inches apart, taking care not to cause bleeding. Old tuberculin is rubbed into one of these abrasions and the other is left as a control. A positive reaction is denoted by the appearance within twenty-four or forty-eight hours of a bright red papule, which later becomes dark red, often reaches a diameter of 10 mm., and persists for about a week.

At the present time the method of choice is the *intracutaneous test of Mantoux,* using O.T., or preferably P.P.D.

When P.P.D. is used the tablet of weaker strength is tried first, then, if there is no reaction, the stronger tablet is employed. In each case the tablet is dissolved aseptically in 1 c.c. of the buffer solution furnished and 0.1 c.c. of this solution is injected intradermally. The dose from the less concentrated tablet contains 0.0002 mg. P.P.D., whereas the stronger one has 0.05 mg., 250 times as much, in one test dose.

Meaning of tuberculin reactions. A positive tuberculin reaction always means infection, but it does not indicate the extent of the tuberculous lesion nor whether the infection is progressing or latent. In adults, the diagnostic value of the test is decidedly limited because so many persons have been infected and give a positive reaction, yet do not have *active* tuberculosis. In children, however, and in young people through the high school ages, a positive tuberculin reaction is often of real significance and a positive aid in diagnosis. Through the health departments in many communities large groups of school children (all who are willing), are given tuberculin tests. Those who reacted positively are then given physical examinations and x-rays are made of the chest. This important health measure has resulted in the discovery of many active cases at a time when recovery, under proper care, can be most confidently expected.

From what has been said above, it would be expected that those persons who have never been in contact with tubercle germs, have never been infected and, therefore, give a negative tuberculin test, would be highly susceptible to tuberculous infection. Some studies on this point have seemed indeed to show clearly that in the case of nurses, for example, active tuberculosis appears more often among those who have a negative tuberculin reaction when they enter training than among those with a positive reaction at that time. It must not be imagined from this that a positive tuberculin test is any guarantee against the liability of acquiring active tuberculosis. Young nurses and doctors, who are often exposed to tuberculous patients, must take care to prevent contact infection, and must avoid habits that predispose to tuberculosis, whether they are tuberculin positive or not.

Prevention and treatment. The prevention of tuberculosis is more of a social, than a medical, problem. Any measures or agencies that relieve overcrowding, and generally improve the living and working conditions of the people, help to reduce the amount of tuberculosis. This is so generally recognized that nearly every community of any size has its tuberculosis society, organized by laymen, to assist health authorities in the control of the disease, and devoted to the promotion of better housing, playgrounds, and many other activities all looking to the same end.

The same influences which prevent the development of active tuberculosis also will *cure* the disease if given a real chance early in its course. *Extra rest*, and good food, fresh air, and sunshine, are the only medicines needed in most cases. Early diagnosis is exceedingly important, in order that the necessary rest treatment may be begun before the infection gets too far advanced. Many communities have established special *tuberculosis clinics* where diagnosis may be made. If a patient is found to have a progressing tuberculous infection, he must be prepared to submit himself to the necessary treatment *for a sufficient period of time* to effect a permanent cure. Under the care of a physician, and aided by visiting nurses or social workers, he may stay in his own home with as much hope of success as he would have anywhere else, in many instances, or he may be sent to a sanitorium. Most communities have facilities for the segregation of advanced cases in special *tuberculosis hospitals.*

It is especially important that children be protected from heavy doses of the germs. Infection through milk is prevented by pasteurization or boiling of all milk, and by the elimination of tuberculous cows from dairy herds. Special care must be provided for the children of tuberculous parents. A *tuberculous preventorium* is provided in many communities, where these children and others who are undernourished and liable to develop the disease may be given a chance to build up their resistance.

Prophylactic vaccination. Many attempts have been made to devise a vaccine which could be used successfully for the prophylactic immunization of individuals against tuberculosis. Very recently it has been shown that a prolonged series of injections of *dead* tubercle bacilli will build up an appreciable immunity in animals. But the practical value of such a vaccine for human immunization is unknown. The late Dr. Calmette of the Pasteur Institute, Paris, has advocated the vaccination of infants born of tuberculous parents with the so-called *BCG* (Bacille Calmette-Guerin) strain of tubercle bacillus. This is a culture of a bovine type which has been cultivated for many years on a glycerin-bile-potato medium, so that it has completely lost virulence for animals. The vaccine is administered in the baby's milk, on alternate days during the first ten days of life. At the present time, it seems to have been shown that this is a safe procedure, but its practical value in preventing tuberculosis is still not fully demonstrated.

LEPROSY

Few diseases have so powerful a hold on popular imagination as leprosy. Though the vast majority of individuals have never seen a leper, nor read an authoritative account of the disease, they are nevertheless quite sure that it is very highly contagious and horrible beyond description. Most people would expect the most dire consequences to follow should one of their neighbors be discovered to be a leper. This universal fear is evidence of the profound impression this disease has made upon men's minds, but like most popular notions of the kind, it is based more upon legend and misunderstanding than truth. Some of the manifestations of leprosy are horrible enough, but cases are now rare in this country, and, contrary to the popular idea, the disease is *not* easily communicated. It occurs most often in persons who have been in long and intimate contact with a leper. Nurses and doctors in leprasariums very rarely contract the disease, and the practice of ordinary personal cleanliness and hygiene protects against infection.

Leprosy has been known since ancient times. In the Middle Ages it was common all over Europe. So great was the fear of the disease that lepers were often treated with a heartless cruelty. The unfortunate victims were made social outcasts, branded "unclean," and forced to beg for the means to sustain their miserable existence. During the fourteenth and fifteenth centuries in Europe, the people began to make deliberate efforts to suppress leprosy by various drastic means, but principally by the segregation of all lepers in special institutions. These measures were so successful that the disease rapidly declined. At the present time, it is almost unknown in central Europe, though still prevalent in Norway and Russia. It is now most common in tropical and subtropical areas. It is found in China, India, and other Eastern countries. Numerous cases occur among the native populations of Hawaii, where the Territorial Government maintains a leprasarium, in the Philippines, and in Mexico. In the United States, there are probably less than 1,000 cases, though the exact number is unknown. Most of the patients are found among immigrants, especially Orientals, and among the native white population of Louisiana, Texas, and Florida. Whenever a case of leprosy is found in the United States, the patient is required to accept the care of the Public Health Service at the National Leper Home in Carville, Louisiana.

Ordinarily, leprosy runs an extremely chronic course. The incubation period is commonly about five years, but it may be as long as twenty years after exposure to the disease before definite symptoms or signs of leprosy appear. There are two principal clinical forms of leprosy; both commonly appear in the same patient. In the *nodular*, or *cutaneous form*, hard nodular swellings develop in the skin, especially on the face, which may greatly distort the features, and the mucous membranes of the nose and throat, the eyes, and sometimes internal organs, such as the liver, are the seat of similar destructive lesions. In the *anesthetic* or *neural form* the germs localize in the nerves, causing loss of sensation in the extremities; the fingers and toes may ulcerate and drop

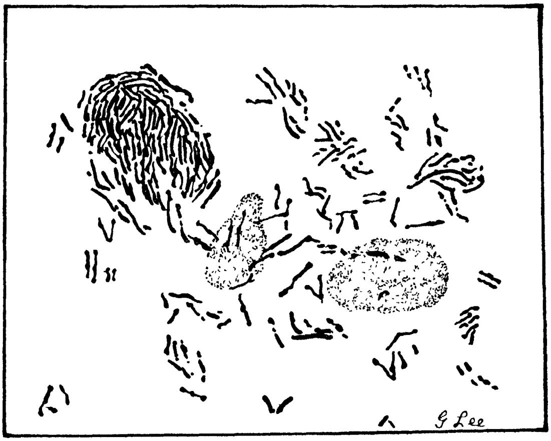

FIG. 89. *Mycobacterium leprae* in a direct smear from a skin lesion of a leper. Drawn from a single microscopic field. In such smears the irregular-shaped, acid-fast bacilli are usually very abundant, often occurring in large ball-like masses. Original preparation by Dr. Florence L. Evans, U. S. Marine Hospital (National Leprasarium), Carville, La.

off, and contractures of the limbs may cause various abnormal postures.

It is not known exactly how leprosy germs are transmitted. The primary infection may occur through the skin, or the nasal mucous membrane. It is certain that the infection is not readily communicated and that most individuals are highly resistant, for a prolonged and intimate contact with lepers seems to be necessary. And even this contact does not lead to infection when ordinary cleanliness and care are exercised. In most parts of the United States leprosy fails to spread, even though (as in New York) infected immigrants may be present in the community.

Mycobacterium leprae. The germ of leprosy is an acid-fast bacillus, resembling in appearance the tubercle bacillus. It was first described by Hansen (1874), in sections of skin from leprous nodules. The organisms are present in enormous numbers in these nodules, lying in bundles between the connective tissue cells, and crowded within the large round cells which make up the nodule (Fig. 89). Numerous attempts have been made to isolate these organisms in laboratory cultures, and bacteria of various kinds, some acid-fast and some not, have been grown by different investigators from leprous lesions. It is still very doubtful, however, whether the true leprosy bacillus has ever been cultivated. The organism is clearly an intracellular parasite.

Laboratory diagnosis of leprosy can be made by microscopic examination, with an acid-fast stain, of scrapings from the nasal mucous membrane or of bits of skin clipped from a leprous nodule. If there is any question that the acid-fast organisms found in such material may be tubercle bacilli, it is only necessary to inoculate a guinea pig. If the organisms are tubercle bacilli, they will cause the development of tuberculosis in the animal, but if they are leprosy bacilli no illness will be produced.

Institutional care of lepers. It is still the best policy to remove patients with leprosy from contact with healthy persons, and place them in leprasaria, where they may receive expert care. Apparently some success has followed the treatment of leprosy in recent years with chaulmoogra oil or its derivatives.

REVIEW QUESTIONS—CHAPTER XXXVII

1. Discuss the prevalence and importance of tuberculosis.
2. Name and characterize briefly the four types of tubercle bacilli. Who discovered the human tubercle bacilli and when?

3. What peculiar staining reaction do tubercle bacilli show? Name other organisms of the same kind. How is this staining reaction usually demonstrated? What is the probable explanation for acid-fastness?
4. Describe the morphology of tubercle bacilli and their appearance in smears of sputum.
5. Describe methods of isolation and cultural characteristics of human tubercle bacilli.
6. Do tubercle bacilli produce toxins? Discuss the resistance of tubercle bacilli outside of the body.
7. How is the human tubercle bacillus differentiated from the smegma bacillus and other harmless acid-fast bacilli? Describe the characteristics of the avian and cold-blooded types of tubercle bacilli and their differentiation from the bovine and human types.
8. How does the bovine type differ from the human tubercle bacillus?
9. What are some of the clinical forms of tuberculosis in human beings? Explain the nature of tubercles and the reaction of the tissues to their presence. How does tuberculous infection come to be *latent?*
10. Discuss the prevalence and importance of latent tuberculosis. Distinguish between tuberculous infection and tuberculous disease. What two kinds of evidence prove the almost universal occurrence of tuberculous infection?
11. Discuss the factors which influence the development of active tuberculosis.
12. Discuss the origin, importance, and prevention of infection of human beings with the bovine tubercle bacillus.
13. Explain the methods employed for the laboratory diagnosis of tuberculosis. When is it necessary to use animal inoculation?
14. How would one proceed to make a laboratory diagnosis of tuberculosis of the kidney?
15. What were Koch's observations on immunity in tuberculosis? What is O.T.? May tuberculin be used in treatment of active tuberculosis? How does the body react to different doses of tuberculin?
16. What is P.P.D.? How are diagnostic tuberculin tests in human beings performed? Define von Pirquet test, Mantoux test.
17. Discuss the value of tuberculin tests in discovering cases of active tuberculosis in man.
18. Is a person who gives a positive tuberculin test probably more, or less susceptible to a new infection? Why?
19. What are the important general measures used in the prevention and treatment of tuberculosis?
20. What is the BCG vaccine?
21. Discuss the historical importance and present prevalence of leprosy. What are the two clinical forms of leprosy? Is the disease highly contagious?
22. Name and describe the causative organism of leprosy. How does it resemble, how does it differ from, the tubercle bacillus? How is its presence in leprous lesions demonstrated?

CHAPTER XXXVIII

INFECTIONS ACQUIRED FROM ANIMALS

IN THIS chapter we shall describe briefly a group of infectious diseases that have a number of features in common. All of them affect animals primarily, though they are often transmitted to man, and in each case there exists among certain animal species a reservoir of the infection which constitutes a perpetual menace to the health of human beings. In the spread of several of these diseases insects play an important part.

BRUCELLOSIS (UNDULANT FEVER)

In a previous chapter we have mentioned that the *Brucella* organisms cause one of the most prevalent and dangerous of the milk-borne diseases—*undulant fever*, or *brucellosis.*

Brucella. The causative organisms of brucellosis are small, somewhat pleomorphic bacilli, only about half a micron wide and little more than a micron long.

They are Gram-negative, nonspore-bearing, and nonmotile; no capsules have been observed. They may be cultivated on plain media at 37° C. but develop better in the richer kinds, such as liver infusion or serum agar.

Three "species" are recognized: (1) *Brucella melitensis*, originally isolated by Bruce in 1886 and shown to be the causative agent in typical Malta fever, which is contracted by drinking *goats'* milk containing these organisms, (2) *Brucella abortus*, originally identified by Bang in 1897 as the causative agent of contagious abortion in cattle, and (3) *Brucella suis*, isolated by Traum from aborting swine in 1914. There are only slight differences between these three varieties, and all are capable of causing undulant fever in man.

Sources of infection. Human beings are always infected secondarily from animals; brucellosis probably never spreads directly from man to man. The germs may gain entrance either through the skin or through the mucous membrane of the gastrointestinal tract. Persons acquire the infection by the ingestion of raw milk from infected cows or goats (or possibly through butter or cheese made from contaminated milk), by direct contact with infected living animals, or by handling infected carcasses or meat. Many bacteriologists have been infected while working with the *Brucella* in the laboratory.

Brucellosis in man. Virulent strains of *Brucella* produce in human beings a specific disease, characterized by periods of fever, sweating, rheumatic pains, and other signs and symptoms of a generalized infection, alternating with periods in which the fever and other symptoms temporarily disappear. The onset of the disease is usually gradual, after an incubation period of two or three weeks. The fever may follow the typical undulating course for weeks or months at a time. Some cases are so mild that the patient is never confined to bed, and eventual recovery is the rule, but often the illness is both severe and prolonged, and the victim is greatly weakened, has an enlarged spleen, and often a marked anemia. In the later stages of the disease the organisms may localize in various sites within the body, as in the joints, brain, or meninges.

Bacteriological diagnosis. The clinical picture of brucellosis in man, especially in mild cases, is often not sufficiently distinctive to permit a definite diagnosis without the aid of the laboratory. The bacteriologist makes (1) *blood cultures,* and performs (2) *agglutination tests with the patient's blood.*

Blood cultures may be made in the usual way by introducing 5–10 c.c. of the patient's blood directly into flasks of liver infusion broth. The *Brucella* grow out slowly, and cultures must be watched for two weeks or more.

Agglutination tests with the blood serum of the patient are relied on for diagnosis in most cases. The general method is the same as in the Widal test for typhoid fever. A series of dilutions of the patient's serum is set up, and each dilution is mixed with a standard suspension of killed organisms (*Brucellus abortus* only is routinely used in the United States). The mixtures are incubated at 56° C. for several hours. A titer of 1:100 or more is significant in clinically suggestive cases; as in typhoid, if the titer can be shown to be rising, the evidence for *Brucella* infection is strong.

Prevention. The control of brucellosis is a complicated and difficult problem. Detection of *Brucella* infection among dairy cattle or other domestic animals by agglutination tests with their blood, and destruction or isolation of infected animals would be valuable measures. For the protection of the general public all milk for direct consumption, or for use in making other dairy products, should be pasteurized. No practicable method has yet been devised to prevent those cases that arise from direct contact with infected animals.

PLAGUE

Plague has been the most destructive pestilence ever suffered by mankind. Great epidemics have swept over most of the world repeatedly. Celebrated accounts of the "Black Death" (as plague was called in the Middle Ages) are given by Boccaccio, and by De Foe in his *Journal of the Plague Year*, and Pepys in his *Diary*. The last extensive epidemic began in Hong Kong in 1894 and spread widely through most of the world. It was during this epidemic in China that the germ of plague, *Pasteurella pestis*, was discovered independently by Kitasato and Yersin. The disease is still common, and terribly destructive to human life, in such countries as India, and China, and other places where sanitary conditions are bad.

Source of infection. Plague is primarily a disease of rats and other rodents. Rats are great travelers, and almost all ships are infested with them. Ships harboring plague-infested rats have carried the disease to practically all the ports of the world. Plague first appeared in the United States in San Francisco in 1900, and more recently cases have occurred in New Orleans, Texas, and Florida. In California there have been several hundred cases, but never an extensive epidemic, due to the energetic work of officers of the Public Health Service. As late as 1924, there occurred an outbreak of pneumonic plague in Los Angeles with several deaths, but since that time cases have been very few. Nevertheless, plague is still a menace there because the ground squirrels, as well as rats, have become infected, and there is always the possibility that a severe outbreak of the disease may occur among these animals with the consequent danger of spread to human beings.

The organisms of plague are carried from rat to rat and from rat to man by fleas. Plague is most likely to occur among those persons who live or work in rat-infested buildings, or who handle dead rats, ground squirrels, or other rodents that might be infected. Among the many varieties of fleas which may carry the germs, the rat flea called *Xenopsylla cheopis* is the most important. Rodents of various species are plague-infected in different parts of the world, and the fleas of any of these animals, though doubtless preferring their usual host, may bite human beings. When fleas bite an infected rat or other rodent, they swallow the plague bacilli, then when they bite a human being, these germs are regurgitated or deposited with feces on the skin. The bacilli are able to invade the body easily through the tiny puncture wound made by the fleabite, or the organisms may be rubbed in by the clothing or by scratching when the fleabite becomes inflamed.

Clinical forms of plague in man. The most characteristic form of plague in human beings is so-called *bubonic plague.* The germs which have entered by means of a fleabite, localize in the nearest lymph glands, which swell and later soften and discharge pus. The femoral glands in the groin are most commonly involved, since fleas often bite the legs. When these glands swell, they are called "buboes"—hence the name bubonic plague. The germs spread to other glands and to the blood stream and in the majority of cases a fatal septicemia results. Sometimes a very acute, fatal blood infection (*septicemic plague*) develops without primary localization in the glands. These forms of plague probably never spread directly from man to man.

A third form of the disease, however, is directly communicable from one person to another, and doubtless cases of this kind always occur in extensive human epidemics. This is *pneumonic plague,* in which the lungs are primarily involved, then later the blood, with death resulting in practically every instance. This infection spreads rapidly by personal contact in the same manner as other respiratory diseases. Sometimes pneumonic plague develops in a case which was originally bubonic, the individual then becomes in the few days of life that may remain to him, a source of infection of the pneumonic type. Patients with bubonic plague sometimes recover, and when they do, they possess a lasting immunity to the disease.

Pasteurella pestis. The organisms of plague are small Gram-negative nonmotile bacilli, usually oval in outline, and staining only at the poles (Fig. 90). In smears from the body they show a narrow capsule. They grow moderately well on ordinary culture media at an optimum temperature of 30°–35° C. In cultures, they have a marked tendency to develop involution forms, of bizarre shapes, particularly when grown on agar containing 3 per cent sodium chloride. They have no spores and are easily killed by heat and the usual chemical disinfectants. They do not form an exotoxin, and, like the germs of anthrax, glanders, and tularemia, they owe their disease-producing power solely to their capacity to invade the tissues and multiply there very rapidly. They are highly pathogenic for laboratory animals. Plague bacilli belong to the group of germs called the hemorrhagic septicemia group, and closely related species are responsible for the common disease called *hemorrhagic septicemia* in cattle, pigs, sheep, guinea pigs, and many other animals.

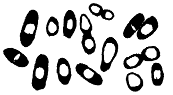

FIG. 90. *Pasteurella pestis,* the cause of plague.

Bacteriological diagnosis. Prompt diagnosis of any case of human plague is of the greatest importance. The plague bacilli may be demonstrated in smears and in cultures from the bubo, or (in pneumonic cases) from the sputum. Sometimes blood cultures will reveal the organisms. Final proof that one is dealing with *Pasteurella pestis* may be obtained by inoculating laboratory animals with the original material or with cultures. The germs are so highly invasive that they will enter and infect a guinea pig if material containing them is merely rubbed on the shaven skin, and a pure culture can be recovered from the organs of the animal.

Prevention. There is little danger that plague will ever reach serious epidemic proportions in this country so long as the present precautions are continued. Health officers in the Pacific states, and elsewhere that plague has appeared, maintain a constant vigilance to detect the possible outbreak of plague among the rodent population, and they are prepared to take vigorous steps to stamp out the focus of infection before the disease spreads to man. Continual warfare is waged against rats and other rodents. At ocean ports, ships are quarantined and fumigated routinely, and efforts are made to prevent rats getting from ship to shore. The extermination of rats everywhere is worthwhile, not only because they are a possible source of plague, but because they cause a tremendous economic loss. As in the control of any other living pest, the most effective measures are those which help to prevent the *breeding* of the animals. The best means of accomplishing this is to make all houses, stores, granaries, ships, etc., rat-proof, i.e., construct them in such a way that rats cannot find safe harborage and cannot readily secure food.

TULAREMIA

This disease occurs naturally in wild rabbits, ground squirrels, and other animals, and may be transmitted to man. In many respects it resembles plague, but is not so often fatal. It is spread from animal to animal by blood-sucking insects, especially ticks. Infected rabbits show numerous small abscesses in the liver and spleen, giving a spotted appearance to these organs.

The germ was first described by McCoy and Chapin in 1912, who found it in infected ground squirrels. They named it *Bacterium tularense*—from Tulare, the county in California where the disease was first observed, and the disease it causes is now called *tularemia.*

The first case of tularemia in man was discovered in the United States in 1914. Many cases have since been reported in almost all parts of the country. Recently the disease has been recognized in Norway, Russia, Japan, and other countries.

Tularemia in man; sources of infection. Horse flies, ticks, and possibly other biting insects that have become infected by feeding upon infected rodents, may carry the germs to human beings. But most cases in the United States have resulted from infection through some slight wound in the skin of the hands while dressing infected wild rabbits. Hunters, cooks, and butchers are especially liable to contract the disease during the rabbit hunting season. In three to nine days an ulcer develops on the hand, or on the conjunctiva, the adjacent lymph glands become swollen and painful, and fever develops which often persists for two or three weeks or more. Recovery is usually very slow, and relapses months or even years later may occur. About 5 per cent of cases are fatal. Like the other organisms described in this chapter, the tularemia bacilli are highly invasive, and numerous cases of tularemia have occurred in bacteriologists who have become infected from laboratory cultures.

Bacterium tularense. The organisms of tularemia are very small, Gram-negative, nonmotile bacilli, having at times a coccoid form, and at other times appearing as tiny rods less than 2 micra long. Capsules probably develop in the tissues. Although usually classified with the plague bacilli in the genus *Pasteurella* (*Pasteurella tularensis*), the tularemia organisms differ from the germs of plague, especially in their cultural requirements. They will not grow on ordinary media, but demand enriched media, such as dextrose blood agar, containing cystine. No toxins have been found. The organisms have no spores and are readily killed by heat, so that thoroughly cooked rabbit meat is safe.

Bacteriological diagnosis. The tularemia bacilli may sometimes be found microscopically in discharges from ulcerating lesions and may be cultured from this material or (rarely) from the blood. Cultures, or the patient's blood, may be used to reproduce the infection in guinea pigs. Diagnosis is usually made, however, by *agglutination tests*, using the patient's blood serum and a standard suspension of killed *Bacterium tularense*. After the first week of the disease (and for months or years after recovery) the blood of most patients will agglutinate tularemia bacilli in high dilution (1:500 or more). The patient's blood should be tested at the same time with *Brucella* organisms, and with plague bacilli, for brucellosis, plague and tularemia may be confused with each other clinically.

Prevention. To avoid danger of tularemia, hunters should beware of rabbits which appear ill, or have been too easily caught. In dressing these animals great care must be taken not to cut the skin of the hands. It would be advisable to wear rubber gloves. Thoroughly cooked rabbit meat, even from an infected animal, is harmless for food.

ANTHRAX

Anthrax is primarily a disease of cattle, sheep, and other animals, from which human beings are infected secondarily. It is caused by *Bacillus anthracis*, a large Gram-positive, aerobic, spore-bearing bacillus. As we have explained in Chapter III, anthrax was one of the first diseases to be studied by the newer bacteriological methods worked out by Koch and Pasteur (1877), and the first human infection for which a particular germ was proven to be responsible. The Latin word *anthrax* comes from a Greek word meaning a *coal* and refers to the characteristic dark, tarry color of the blood of animals which have died from the disease.

In animals, anthrax usually takes the form of a septicemia, and death often occurs within a few days. The germs remain almost entirely within the blood vessels, but become so numerous there that the capillaries are literally choked with them. The disease does not spread from animal to animal, but is contracted by infection with the spores of the germ through the intestinal tract from grazing on contaminated pasture land or from eating artificial foodstuffs containing the spores. When an animal dies the anthrax germs escape to the ground with blood and excretions from the body. In the presence of oxygen outside of the body, the bacilli are converted to the spore form. These *anthrax spores* are extremely resistant, and may remain alive for years upon the surface of the ground, particularly in the moist soil of low, swampy regions. When cattle or sheep graze upon such land, they may swallow these spores, or the germs may enter through breaks in the mucous membrane of the mouth.

Anthrax in man; sources of infection. Human anthrax is always contracted either directly from animals, or from animal products containing the spores. Two groups of persons are liable to anthrax infection. These are: (1) shepherds, farmers, butchers, veterinarians, and others, who come into close contact with infected animals, and (2) workers in industries concerned with the handling of animal products such as wool, hair, hides or skins. In the latter group, infection occurs through the extraordinarily resistant anthrax *spores* which cling to the hide or hair, or similar material, throughout many steps of manufacturing processes. Indeed, so long as the material is not sufficiently heated or otherwise disinfected, the spores may remain alive and virulent, and they are able to invade the skin very easily through the slightest of abrasions. Several cases are known in which infection of the face followed the use of unsterilized shaving brushes made from contaminated hairs.

When anthrax spores get into the skin of human beings through abrasions, they cause a characteristic local boil-like lesion known s a *malignant pustule.* Man is less susceptible to the germs than animals, so that the infection usually remains localized, and recovery is the rule. When death occurs, there is a terminal septicemia. In workers who handle wool, pulmonary anthrax, or *woolsorter's disease,* sometimes occurs. This severe, and often fatal infection is contracted when large amounts of dust containing anthrax spores are inhaled. Rarely, there may occur an *intestinal* form of *anthrax,* caused by eating food contaminated with the spores.

Bacillus anthracis. The anthrax organisms are large Gram-positive, square-ended rods. Capsules about the organisms may be seen in smears from infected tissues. In cultures the bacilli form long chains; the oval, centrally placed spores develop while the organisms remain in chain formation (Fig. 91). They grow readily on ordinary culture media, and in both morphological and cultural characteristics closely resemble *Bacillus subtilis* and other nonpathogenic, saprophytic, spore-bearing bacilli. They are distinguished principally by the fact that they are *nonmotile,* whereas most saprophytic species are motile, and they are *highly pathogenic for* guinea pigs and other *laboratory animals,* while the saprophytes are harmless.

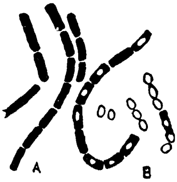

FIG. 91. *Bacillus anthracis,* the cause of anthrax. A, vegetative forms in the characteristic chain arrangement; B, sporulating forms and free spores.

Bacteriological diagnosis. The diagnosis of anthrax in man is made by microscopic examination of material from pustule or sputum, by study of cultures, and by pathogenicity tests. The bacilli grow out readily on plain agar. Blood cultures may yield a growth of the organisms in some cases. The final step to prove the presence of *Bacillus anthracis* in any material is to inoculate a guinea pig or mouse. The animal will die in about forty-eight hours and the black blood will swarm with anthrax bacilli.

Prevention. *General measures.* To protect industrial workers from anthrax infection, all materials such as hides, hair, and wool, which might possibly be contaminated with anthrax spores, are now thoroughly disinfected, and manufactured products, such as shaving brushes are sterilized.

It is now the practice almost everywhere to exercise great care when an animal dies of anthrax to prevent contamination of the ground. The animal is never skinned, the carcass is not opened, and every effort is made to avoid spilling blood. The carcass is completely burned, or buried deep and covered with quicklime.

Prophylactic vaccination of animals. In regions where anthrax is continually occurring among cattle and sheep, because the pasture land is contaminated with spores, the only successful method of controlling the disease is to vaccinate all the animals periodically. The method still most widely employed, though many modifications have been suggested, is that introduced by Pasteur in 1881. Two vaccines are used: (1) a subculture in broth from a strain of anthrax bacilli incubated at 42°–43° C. for fifteen to twenty days, so that it no longer forms spores, and has lost its virulence for guinea pigs but will still kill mice, and (2) a subculture from a strain kept at the same temperature for only ten to twelve days. This second vaccine is much more virulent than the first; it is fatal for guinea pigs but not for rabbits. Cattle and sheep are given an inoculation of vaccine (1), then, about twelve days later, a smaller dose of vaccine (2). The protection conferred by this process of active immunization lasts for only about a year, and to keep animals permanently immune the vaccination must be repeated annually. Wholesale vaccination of herds of cattle and sheep has been carried out in many countries with good results.

Serum treatment of human anthrax infection. Immune serums, prepared by immunizing sheep or horses against anthrax bacilli, have proven of value in the treatment of human anthrax, particularly in those cases in which the organisms appear in the blood stream and there is grave danger of fatal septicemia.

GLANDERS

Glanders is primarily a disease of horses which is occasionally transmitted to man. It is caused by *Loefflerella mallei*, a small Gram-negative bacillus, first isolated by Loeffler and Schütz in 1882.

In horses the disease may appear in two forms, called *glanders* when the infection involves the internal parts of the body, especially the nasal passages and lungs, and *farcy* when the disease is characterized by swellings and ulcerations of the skin and adjacent lymph glands. The infection spreads to other animals by direct contact.

In man glanders is not common, but when it does occur it is usually severe, and almost invariably fatal. It attacks chiefly those persons whose work brings them in close and continual contact with horses. The organisms invade the body through some break in the skin, or by way of the nasal mucous membrane, following direct contact with a germ-laden discharge from an infected animal. The acute form of glanders in man is characterized by fever, nasal discharge, and skin eruption, and is usually terminated by death within a week. Numerous cases of glanders have occurred among laboratory workers from handling cultures of *Loefflerella mallei.* There are very few other bacteria so dangerous for the bacteriologist.

Loefflerella mallei. This organism, the specific cause of glanders, is the same as is widely known as *Pfeifferella mallei.* It is a slender, Gram-negative, nonmotile rod. It grows rather poorly on laboratory media when first isolated. Its most characteristic growth is developed on potato media where it forms a brownish, honey-like mass. It is easily killed by heat and the ordinary chemical germicides. When the organisms are inoculated intraperitoneally into a guinea pig, there occurs within a few days an intense congestion and swelling of the testicles, the so-called *Straus reaction.* The bacilli may be cultured from the affected testicles.

Bacteriological diagnosis. Diagnosis of acute glanders may be made by microscopic examination of the discharge from fresh lesions, and by cultures made on agar and potato media. Guinea pigs may be inoculated to elicite the Straus reaction.

Chronic or latent glanders in horses is diagnosed by the *mallein* test, which is in every respect comparable to the tuberculin test used in tuberculosis. *Complement-fixation tests,* using suspensions of *Loefflerella mallei* as antigen, are successful in the diagnosis of glanders in either horses or human beings.

TYPHUS FEVER AND ROCKY MOUNTAIN SPOTTED FEVER

These are the two most important of the diseases caused by *Rickettsia* (see Chapter VII). They are appropriately mentioned in this chapter for, like the bacterial infections discussed above, animals are the primary source of the disease in man, and insects play the determining rôle in their spread.

Typhus Fever now exists in two distinct forms: (1) *European, or epidemic typhus,* the classical typhus fever of olden times, transmitted from man to man by the body louse, and (2) *endemic* or *murine typhus,* which is a natural infection in rats and is carried to man by the rat flea. Both are caused by the species of rickettsiae called *Rickettsia prowazeki.*

European or epidemic typhus. This ancient malady has played a conspicuous part in the gloomy history of epidemic disease. Typhus is one of those pestilences with a high mortality that plagued European and Asiatic peoples for centuries before general living conditions and standards of personal cleanliness improved sufficiently to limit their spread. This louse-borne "jail-fever" (to give it one of its common names), was constantly associated with war, famine, overcrowding, and filth. In modern times it has greatly diminished and is now restricted to relatively small areas. In Europe, a principal focus remains in Russia and the Balkan States. In North America the disease occurs in parts of Mexico, but has never found a permanent foothold in the United States.

Endemic or murine typhus. This form of typhus infection is more widespread, and occurs with special frequency in seaports the world over. It is prevalent in Mexico, and several hundred cases have been reported within a single year in the United States along the Mexican border and in communities along the Atlantic seaboard. The disease is much milder than the classical European typhus, having a death rate of only about 5 per cent. Clinically it resembles typhoid fever, and also the milder cases of another *Rickettsia* disease, Rocky Mountain spotted fever, with either of which it may be confused. There is a high and continuous fever and a more or less characteristic skin rash.

Endemic typhus is referred to as typhus of the *murine type* because it is primarily an infection of rats. Rat fleas and rat lice carry the causative rickettsiae from rat to rat. As in plague, human beings are infected when feces of infected rat fleas are rubbed into the skin about the fleabite. The rickettsiae multiply actively in the fleas, but do not harm the insect. (In contrast, human body lice that transmit the European typhus from man to man themselves invariably succumb eventually to the rickettsiae they harbor.)

Rocky Mountain Spotted Fever. Until about 1930 it was generally believed that this disease was confined to the Rocky Mountain region, especially Montana and Idaho, with occasional cases in other western states. In that year, however, it was established that a form of the infection occurs also in Minnesota and in the eastern United States. Clinically similar diseases long known in other regions of the world, in particular a form of "typhus" occurring in Brazil, are now recognized as varieties of this same infection.

The typical Rocky Mountain spotted fever of Montana is a severe disease, with a mortality rate as high as 80 per cent. The eastern form of the infection is milder, having a mortality of about 25 per cent. There is a rash which suggested the name "spotted fever."

As we have previously explained it is the common *wood tick* of the western United States (*Dermacentor andersoni*) that acts as the insect carrier of the Rocky Mountain spotted fever rickettsiae. The organisms survive in the eggs of the ticks and pass into the newly hatched ticks, so that the parasite is perpetuated without the necessity for a mammalian host. In the eastern states the dog tick, *Dermacentor variabilis*, is the vector.

Rickettsia rickettsi is the name most commonly used for the rickettsiae of spotted fever.

Laboratory diagnosis of typhus fever and Rocky Mountain spotted fever. It is not practicable to attempt to demonstrate the causative rickettsiae microscopically. Sometimes guinea pigs are inoculated with some of the patient's blood, but the symptoms and pathological changes that may develop in these animals require for their proper interpretation more expert knowledge than is ordinarily possessed by the laboratory worker. For routine diagnosis of rickettsia infection the most useful procedure is the *Weil-Felix agglutination test with the patient's blood.*

This test depends upon the fact that *the serum from patients with typhus and other rickettsia diseases will agglutinate certain strains of Proteus bacilli.* The particular strain of these organisms almost universally used for this reaction is called *Proteus X19.* It was originally isolated by Felix and Weil in 1916 from the urine of a typhus case, though there is no reason to believe that it has anything to do with the causation of typhus. Agglutinins for this organism appear in the blood of most patients within the first four or five days, or at least within the first week of illness. When a positive agglutination is observed in a dilution of 1:320 or 1:640 this is strongly indicative of rickettsia infection, although often the titer runs much higher.

Prophylactic vaccination against Rickettsia infections. One attack of typhus fever or Rocky Mountain spotted fever establishes a high degree of immunity. A number of effective preparations have been devised for producing an active immunity artificially. As a vaccine for Rocky Mountain spotted fever, Spencer and Parker made an emulsion of infected ticks by grinding them up in salt solution containing phenol, and found that this preparation would immunize both man and laboratory animals against the disease. Others have made vaccines from the rickettsiae obtained from the tunica vaginalis of infected guinea pigs. For immunization against typhus, Zinsser and colleagues have prepared vaccines from suspensions of rickettsiae grown in the peritoneum of rats whose resistance to the organisms was lowered prior to injection by treatment with x-rays. At the present time vaccination against both typhus and Rocky Mountain spotted fever is being tried with material from tissue cultures of rickettsiae.

REVIEW QUESTIONS—CHAPTER XXXVIII

1. Name and describe the three varieties of bacteria that may be responsible for brucellosis in man, and explain when and by whom they were first isolated.
2. What is a prominent feature of *Brucella* infection in animals?
3. Describe how human beings may be infected.
4. What is the usual course of brucellosis in man?
5. Outline the practical methods used in making a bacteriological diagnosis of brucellosis in man.
6. What are the principal measures that may help to prevent brucellosis?
7. Discuss the prevalence and importance of plague. What is the origin of human plague? How is the infection transmitted?
8. What are the principal forms of plague in man? How are human beings infected?
9. How may a laboratory diagnosis of plague be made? Name and describe the causative organism.
10. Discuss the prevention of plague.
11. Describe briefly the nature of tularemia, its occurrence in man, sources

of infection, bacteriological diagnosis, and prevention. Name and describe the causative organism.

12. What is the cause of anthrax? By whom was this organism first studied and when? What does the word "anthrax" mean?
13. Describe anthrax in animals. How do animals become infected?
14. What are the principal forms of anthrax infection in man? How does infection occur?
15. Name and describe the anthrax bacillus. How may a bacteriological diagnosis of human anthrax be made?
16. Discuss prevention of anthrax in animals, in man. Describe a widely used form of anthrax vaccine. Who developed this vaccine and when?
17. Is a specific treatment available for human anthrax?
18. Discuss briefly the nature of glanders in animals, its occurrence in man, and methods of bacteriological diagnosis. Name and describe the causative organism.
19. What are the two principal forms of typhus infection in man? How is each transmitted? What is the importance of endemic, or murine typhus? Name and describe the causative organism.
20. Describe the nature and prevalence of Rocky Mountain spotted fever. How is this infection acquired by man? Name the principal insect vectors. Name the causative organism.
21. What procedures are used in the practical laboratory diagnosis of *Rickettsia* infections? Describe the method of testing for the Weil-Felix reaction. What strains of *Proteus* bacilli are used? Is this reaction of positive value in practical diagnosis?
22. How may prophylactic vaccination against Rocky Mountain spotted fever and typhus fever be accomplished?

CHAPTER XXXIX

IMPORTANT VIRUS DISEASES

THE GENERAL properties of filtrable viruses have been described, and a list of the principal virus diseases has been presented in Chapter VII. Special mention of some of the human virus diseases, such as those principally affecting the skin, respiratory tract, and genital regions, has been made in other chapters. Here we shall discuss some important generalized virus infections of man and those primarily involving the central nervous system.

SMALLPOX

Smallpox, or *variola*, has been known throughout history as one of the great scourges of mankind. It was prevalent in ancient times, and swept in a series of devastating epidemics over medieval Europe. It has attacked men of all races and all ages everywhere. In our own time it is still very prevalent in the Oriental countries, but, due to the almost universal practice of vaccination, it is now a comparatively rare disease in most civilized lands.

Clinical forms of smallpox; vaccinia. Before the days of vaccination, which did not become general until the early part of the nineteenth century, smallpox was not only exceedingly common, but very severe, and fatal in a high proportion of cases. Practically everyone had the disease at one time or another, usually during childhood, and it is said that parents did not count a child a member of the family until it had survived its expected attack of smallpox. The patient with virulent smallpox presents a most hideous appearance (Fig. 92). He suffers from fever, headache, and backache, and his body becomes covered with loathsome pustules. If he is lucky enough to recover, he is marked forever with unsightly scars. In the United States and other countries where vaccination has been widely practiced, smallpox in late years has been mostly of a mild type, but there is evidence that the disease tends to become malignant whenever it gets an opportunity to spread rapidly among susceptible persons.

The disease known as *alastrim* which occurs in the West Indies and in other tropical regions, is generally regarded as a mild form of smallpox.

The localized infection produced in the skin of man by inoculation of *cowpox* material, as in vaccination, is called *vaccinia.* There is general agreement that the virus of vaccinia (vaccine virus) is nothing more nor less than smallpox virus which has become permanently modified in virulence for man by adaptation to animal (cattle) tissues.

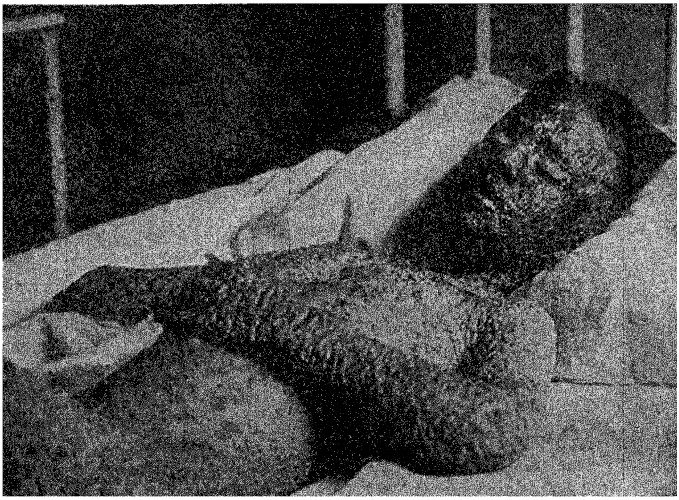

FIG. 92. A severe case of smallpox.

Spread of smallpox. The virus of smallpox is present in the secretions of the nose and throat, in the fluid of the vesicles in the skin, and in the scabs or crusts from the skin lesions. It may also be present in the feces or other body discharges. The infection spreads with remarkable ease and rapidity, and seems to attack practically every individual who is not immune. The usual incubation period is about twelve days. Probably the mode of spread of the virus is principally by personal contact through the nose and throat discharges, but it may be that the infection is transmitted in more remote ways.

The virus of smallpox and vaccinia. Smallpox virus may be recovered in filtrates of nasal secretions and other materials, including the blood, from variola patients. It may be cultivated in tissue cultures and on the chorio-allantoic membrane of the chick embryo. Inoculation of laboratory animals does not reproduce the typical clinical picture of smallpox, but does cause local lesions which are similar to the skin lesion of smallpox in man.

When *variola virus* is inoculated into calves or rabbits and is then passed through a series of these animals, it becomes converted permanently into *vaccinia* (cowpox or vaccine) *virus*, and if now introduced into the human skin it produces only the local lesion familiar as the vaccination sore.

It is now well established that the characteristic inclusions which appear in tissue cells affected by either smallpox or vaccinia virus, first described by Guarnieri in 1894 and known as the *Guarnieri bodies,* represent intracellular aggregations or colonies of the active virus particles. These particles, or elementary bodies, in this particular case are called *Paschen bodies;* there is every reason to believe that they constitute the actual causative agent, the virus itself (Fig. 23). The elementary bodies of vaccinia are among the largest of the viruses, averaging about 150 mμ (0.15 μ) in diameter, though some may reach 250 mμ (0.25 μ) (Fig. 25).

Immunity to smallpox; prophylactic vaccination. *Inoculation with smallpox as a means of immunization.* It has been common knowledge for centuries that recovery from an attack of smallpox renders the individual immune from subsequent attacks. In China, Turkey, and other countries, long before the present method of vaccination was worked out, it was the common practice to *inoculate* healthy persons deliberately with smallpox matter from a mild case, in the hope that the inoculated individual would have a light attack of the disease and so become immune. In 1717, Lady Mary Wortley Montagu introduced the practice of smallpox inoculation into England. It was, of course, an extremely dangerous procedure, and many of the inoculated persons had an attack of cypical, severe smallpox, instead of the mild infection they desired, and some died. Furthermore, the person with inoculation smallpox was just as likely to spread the infection as one who had contracted the disease in the natural way, and while the practice of inoculation did succeed in making some individuals immune, it was also responsible for keeping smallpox more prevalent among the population than it might have been otherwise.

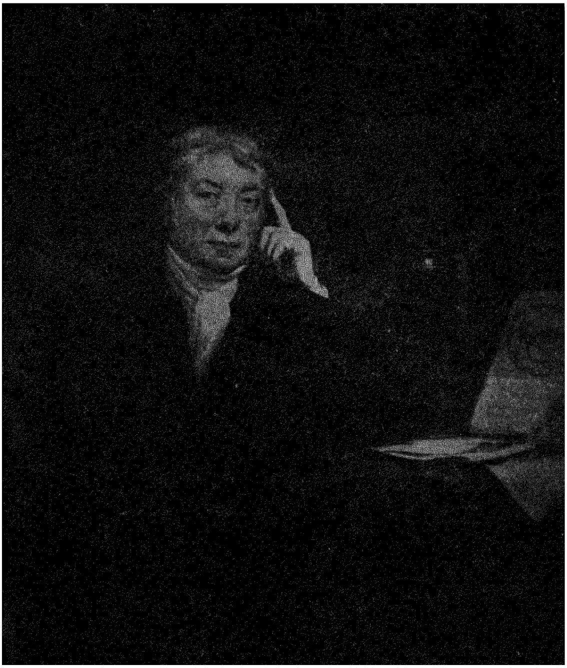

FIG. 93. Edward Jenner, 1749–1823.

Jenner's discovery of vaccination. Credit for the discovery of a safe and efficient method of immunization against smallpox belongs to Edward Jenner (1749–1823), an English country physician (Fig. 93). Jenner developed the method of *vaccination* against smallpox, which consists in the introduction into the human skin of the virus of *cowpox,* or *vaccinia.* Cowpox is a disease which in Jenner's time commonly appeared in dairy cattle in the form of pustules on the teats. Similar lesions sometimes developed on the hands of persons who milked infected cows. These cowpox sores resembled closely the lesions of smallpox, but they remained localized, and the individual experienced general symptoms of only the mildest character. Cowpox did not spread to other persons. It was a matter of common observation among country people that a person who had had cowpox would not take smallpox, and the inference was drawn that cowpox, a very mild, localized infection, must furnish immunity to the more serious disease smallpox.

As a student, Jenner was impressed with these beliefs, and as soon as opportunity was afforded him, he began to study the question very thoroughly. After several years of investigation, he was convinced that cowpox does, in truth, protect against smallpox, and he determined to put the matter to the test. In 1796, Jenner took some of the fluid from a typical cowpox lesion on the hand of a dairy-maid, Sarah Nelms, and transferred it to the skin of a boy, James Phipps. The boy had what we would now recognize as a typical vaccination "take." Several weeks later, Jenner inoculated this same boy with matter taken directly from a pustule of a smallpox patient, but no disease resulted. The boy was immune. Later Jenner made other experiments and collected additional data, all of which supported the claim that inoculation with cowpox is a safe and certain way of producing immunity to smallpox. This supremely important discovery marks one of the most significant advances ever made by man toward the conquest of disease. Though vaccination met with much opposition at first, and has always been opposed by some ignorant or misguided persons, it has stood the test of time, and proven its value over and over again.

Preparation of smallpox vaccines. During the early days of vaccination, it was customary to inoculate human beings with material taken from the pustules of persons previously vaccinated. This practice of arm to arm vaccination sometimes led to undesirable complications, and it has long since been abandoned. The vaccine is now prepared by inoculating healthy young calves, and individual doses of the purified virus from the lesions on these animals (vaccine virus) are made up to be used for the vaccination of human beings.

For the original inoculation of the calves, virus obtained directly from human vaccination vesicles is sometimes employed.

By another method, virus from human vaccination lesions is first inoculated into a calf, and then into rabbits, and the pulp from the rabbit lesions is then emulsified in glycerin and used for the vaccination of calves to produce the regular supply of vaccine for human vaccinations. Young calves known to be free of disease are selected, thoroughly cleaned and washed, and the abdominal surface is shaved and disinfected. A number of scratches are made across the abdomen and into these scratches the virus is rubbed. The vaccinated animals are kept in specially constructed stalls, and every effort is made to keep their surroundings as clean as possible. On the eighth day, when the characteristic pustules are well formed, the vaccinated area is washed with sterile water, and the crusts are removed (Fig. 94). The soft pulp remaining in the lesions is then collected with a sterile curette, transferred to a sterile container and mixed with a 50 per cent solution of glycerin containing a small amount of carbolic acid.

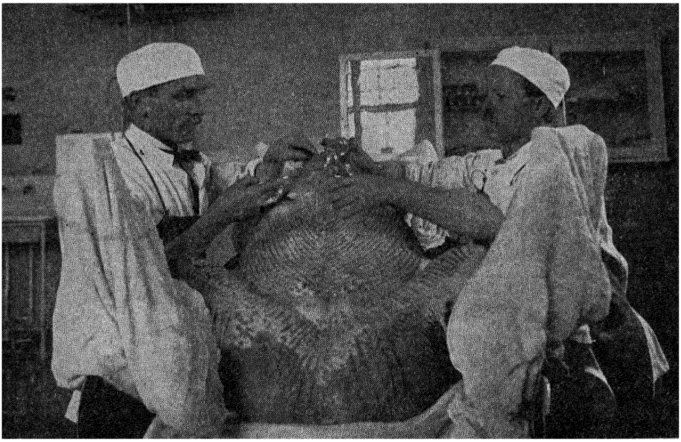

FIG. 94. Collecting the vaccine lymph, containing cowpox virus, from the eruption developed on the calf six or eight days after inoculation with the virus. The lines of inoculation are clearly shown. This lymph, after mixture with glycerin, and storage until tests show it to be free of ordinary bacteria, constitutes the material used for the vaccination of human beings against smallpox. (Courtesy of Parke, Davis & Co., Detroit.)

This glycerinated pulp is then stored in the cold for several weeks. During this time any bacteria in it die off, while the vaccine virus remains active. Cultures are made from the material repeatedly until it is certain that no living bacteria remain, and tests by animal inoculation are made to exclude the possibility that the virus of foot and mouth disease or tetanus germs might be present. When the vaccine is thus proven to be safe, its potency is tested by inoculation of rabbits. Then the vaccine is put up in amounts sufficient for a single vaccination in small, sterile capillary tubes with a rubber bulb attached. Throughout the entire process of manufacture strict aseptic precautions are observed and the vaccine is never touched by human hands. The virus will remain active for at least three months, *provided it is kept continuously cold.*

Rivers has shown that vaccinia virus cultivated in tissue cultures may be safely inoculated intradermally into human beings, and produces a strong immunity comparable to that developed after vaccination with the usual calf vaccine. Goodpasture and associates have described a practical method of making a vaccine from vaccinia virus grown on the chick embryo membranes. It is probable that in the future these newer forms of smallpox vaccines will in part, at least, replace the calf vaccine now used.

Modern methods of vaccination. It was formerly the custom to vaccinate human beings by rubbing the virus into a large scarified area of the skin, but this produced unnecessarily large sores, which were liable to contamination, and left large, ugly scars. In modern methods of vaccination, the insertion of the virus is restricted to a *very small area;* the resulting lesion is smaller, but just as effective in producing immunity.

The skin of the upper arm over the deltoid muscle is washed with soap and water, then with *acetone,* which is allowed to evaporate. The tip of the capillary tube containing the vaccine is held in a fold of sterile gauze and broken, and the virus is expelled on to the prepared skin. Then with the side of a sterile needle, held almost parallel to the skin surface, a number of minute pricks are made into the superficial skin through the drop of virus. This is the so-called *multiple pressure method.** The *scratch method,* in which the virus is rubbed into a single scratch in the skin *not more than one-eighth inch long,* is also permissible.

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* Leake, J. P. "Questions and Answers on Smallpox and Vaccination." *U.S. Public Health Reports,* Reprint No. 1137, 1927.

With either method care must be taken to avoid drawing blood. In a minute or two the excess vaccine is gently wiped off with sterile gauze. No dressing at all need be used, though often the area is covered with a loose fold of gauze.

There is no longer any reason why anyone should be exposed to the danger and inconvenience of vaccination on the leg. By the modern technique a very small area only of the superficial skin is inoculated, and the resulting scar is no more than a "sanitary dimple," not unattractive in appearance. Vaccination should be thought of as a minor surgical operation, and should be performed only by persons who understand aseptic methods.

Local reactions to vaccination and their significance. Provided the vaccine used is potent, and properly applied, *there is always a local reaction of some kind following vaccination.* Failure of the vaccination to cause any reaction whatever is most commonly due to faulty technique, or to the fact that the vaccine used was not fresh, or had been allowed to get warm, so that the living virus originally present in it had been destroyed. Three types of reactions following a successful vaccination may be recognized: (1) *primary vaccinia* (the typical "take"), (2) the *vaccinoid* (or accelerated) reaction, and (3) the *immune* (or immediate) reaction (Fig. 95).

Primary vaccinia occurs in individuals who have never been previously vaccinated, and have not had smallpox. During the first three days, nothing is to be seen, then a papule appears, which by the seventh day becomes a raised, whitish vesicle. This enlarges somewhat during the next few days, but by the twelfth day it begins to dry up, and a crust forms over it, which drops off during the third or fourth week, leaving a characteristic scar. An area of redness (erythema) in the skin about the vesicle, which is at first narrow, begins to spread rapidly about the seventh day, and reaches a maximum diameter of about 6 inches on the ninth or tenth day. At this time there may be also a general reaction, with some fever, and malaise, lasting for a few days.

The *vaccinoid* reaction occurs in persons who have a partial immunity—for example, in persons vaccinated eight or ten years previously. The reaction is less severe, and accelerated, i.e., it develops more quickly, reaches its maximum sooner, and disappears more rapidly. The height of the reaction occurs usually on the fourth to the eighth day, instead of the ninth or tenth

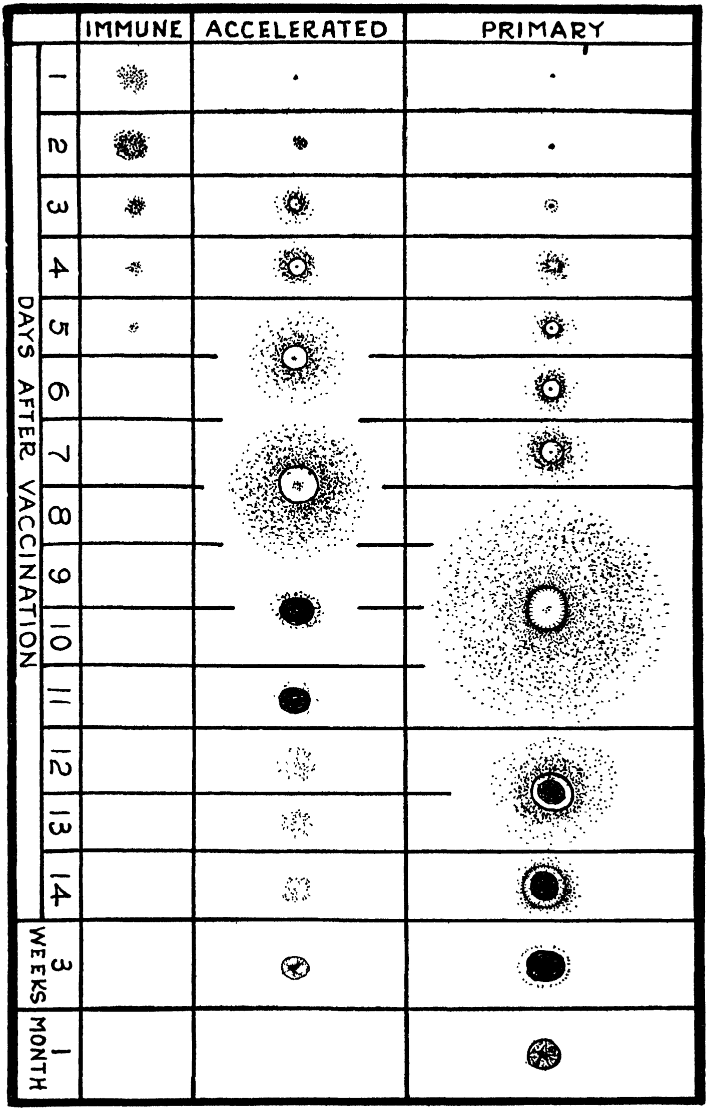

Fig. 95. Types of local reaction which may follow vaccination against smallpox The dotted parts represent the area of redness in the skin about the vaccination site.

day. This is really an allergic reaction, due to the fact that the tissues are in some degree hypersensitive to the virus.

The *immune reaction* occurs in individuals who have a relatively high immunity as a result of recent vaccination or recent recovery from smallpox. It is this reaction which is often mistaken for a failure of the vaccine to take, for unless the arm is examined on the second or third day after vaccination, the reaction may be missed entirely. The immune reaction consists of a small area of redness, reaching its maximum in about forty-eight hours, and possibly a small papule, but there is no vesicle and no diffuse area of erythema as in reactions of the other types.

Care of the vaccination wound. It is important that the vaccination sore be properly cared for, especially in cases of a severe primary take. Like any other wound, it may become infected, and to this is due the occasional serious complications of vaccination. It is best to use no dressing at all, and *no shield of any kind,* and tight binding about the vaccination site should never be permitted. The vesicles produced by modern vaccination methods are small and tough, and will usually dry without rupturing. If necessary to prevent soiling of the clothing, the sore may be covered by a loose fold of sterile gauze. Care must be taken not to contaminate the fingers with the virus, for then it may be reinoculated by rubbing it into the eyes or nose, or into other places on the skin, with embarrassing if not serious results.

The value of vaccination for the prevention of smallpox. The safety of the smallpox vaccines and the prophylactic value of the vaccination procedure have been demonstrated beyond a doubt, and can be denied only by those who wilfully refuse to believe the evidence. The student should consult larger textbooks and references given in this book for specific examples. Analysis of the records of many years show the following significant facts. The amount of smallpox in any country has always declined abruptly when vaccination has been introduced and widely practiced. At the present time the prevalence of the disease in any locality is in direct proportion to the completeness with which the population is vaccinated; in those states and cities of the United States where compulsory vaccination is enforced, the disease is practically nonexistent; whereas, in other communities where vaccination laws are lax, serious outbreaks occur repeatedly. In these epidemics the vast majority of cases (about

95 per cent) are in individuals who have never been vaccinated, a small proportion (about 3 to 7 per cent) occur in persons vaccinated more than seven years previously, and not more than 1 or 2 cases in a hundred occur in those who were vaccinated within seven years' time. Deaths of persons recently vaccinated are extremely rare. That a certain number of cases of smallpox should occur among vaccinated persons is not in the least surprising, and does not lessen the value of vaccination in general. Absolute immunity in every individual following vaccination, or for that matter, following an attack of natural smallpox itself, is not to be expected. Immunity to any infectious disease to which human beings are naturally susceptible is always *relative*, and never absolute. As a matter of fact, smallpox vaccination comes as near to giving 100 per cent protection as any known method of active immunization.

Every infant should be vaccinated when a few months old. The vaccination should be repeated at the school age, and at any subsequent time when there is danger of exposure to smallpox.

Serum treatment of smallpox with serum from convalescent smallpox patients or with an antivaccinia serum prepared in animals is possible, and may be of value.

RABIES (HYDROPHOBIA)

Rabies is an acute and highly fatal disease of animals, most common in dogs and cats, and in wolves and other carnivorous wild animals, but transmissible to human beings. It is caused by a *filtrable virus*, which is present in the saliva of rabid animals. Most cases in man follow the bite of a rabid dog or cat. Rabies is prevalent among dogs, and occasional human cases occur in most countries, except in England, where laws requiring the muzzling of dogs, and strict supervision over imported animals, have been rigidly enforced, with the result that rabies has been practically eradicated there.

Rabies in dogs. It is principally the homeless, stray dog that keeps rabies infection alive among the canine population. Such an animal may bite, and so infect, many other dogs before it succumbs. The disease in dogs begins gradually, two to eight weeks after infection has occurred. The virus may be present in the saliva as much as five days before the onset of definite symptoms. The animal is at first merely restless, and perhaps more affectionate than usual, then the restlessness increases, the animal barks and howls in a peculiar manner, begins to snap at objects about it, and soon becomes furiously "mad." In this stage the unfortunate creature runs aimlessly about, frothing at the mouth, and biting viciously at man or beast or anything else in its path. Finally paralysis sets in, beginning usually in the hind legs, and within a short time the animal is dead. Sometimes a rabid dog shows paralytic symptoms from the first ("dumb rabies"), and does not pass through the furious stage. If an animal suspected of having rabies is securely confined so that it can be safely observed, and symptoms of the kind described above develop, there is no doubt as to the diagnosis. Death will always occur before the tenth day, and usually within five days.

Diagnosis of rabies in animals. *Demonstration of Negri bodies.* The experienced veterinarian has little difficulty in recognizing rabies in dogs or cats from the symptoms and manner of death, and it is important that an animal suspected to have the disease be captured and watched carefully to note these clinical evidences. When a supposedly rabid animal dies or is killed, it is possible to determine whether or not it was really rabid by examination of the brain. In 1903, Negri described certain round or angular bodies which he found within the brain cells and their processes in cases of rabies. These so-called *Negri bodies* (Fig. 22) have since been shown to be a constant and characteristic finding in this disease. To detect Negri bodies smears are made directly from certain portions of the gray matter of the brain, stained with Giemsa or some special stain and examined under the microscope, or a portion of the brain (cerebellum) is hardened and histological sections are made, appropriately stained, and examined. When these Negri bodies are seen, this is definite proof that the animal was rabid.

Animal inoculation. In case Negri bodies are not found, a final test is to inoculate an emulsion of the brain tissues intracerebrally into a guinea pig. If the inoculated material contains the rabies virus, this animal will come down with the disease in about three weeks.

Rabies in man. *Factors of infection.* Not every person who is bitten by a rabid animal will develop hydrophobia. (This fact explains why the application of a "mad stone" to the bite, and other fantastic remedies based upon superstition, have often apparently succeeded in preventing the disease.) Much depends upon the location of the bite, whether it occurred on the bare skin or through the clothing, and the depth and extent of the wounds. Most of the saliva may have been caught on the clothing. The most dangerous type of bite is one through the bare skin which causes a deep or lacerated wound. The importance of the location of the bite will be clear when it is understood that the virus is *neurotropic,* that is, it has a strong affinity for the nervous tissue. *It travels slowly along the nerves to the brain,* and when the central nervous system is reached the symptoms of rabies appear. Naturally, then, a bite upon a part of the body which is abundantly supplied with nerves, such as the hands, is liable to lead to the disease, and the nearer the bite to the brain the shorter will be the incubation period. Bites on the face are, therefore, especially dangerous.

It must be remembered that there is always a possibility of infection whenever the saliva of a rabid animal has come in contact with the broken skin, and the question of taking the "Pasteur treatment" (prophylactic immunization) described below must be seriously considered in every case of such contact, though the animal did not actually bite.

Symptoms. In human beings rabies is invariably fatal. Once the symptoms have begun there is no available treatment which will save the unfortunate victim from a horrible death. Beginning with depression and irritability and difficulty in swallowing food, there follow two or three days of intense excitement, then paralysis ending in death. The patient's fear of water (hydrophobia) is due to painful reflex spasms when he attempts to swallow fluids.

The long incubation period. The fortunate feature of rabies in man is the long period after the virus has been introduced before symptoms begin. This incubation period is rarely less than four, and is usually six to eight weeks or more. Cases developing rabies five or six months, and *rarely* as much as eleven months, after infection, have been reported. It was Pasteur who developed the lifesaving method of giving each person who has been exposed to a possible rabic infection a series of treatments with a vaccine, by means of which an active immunity to rabies virus is built up during the incubation period, and the disease is effectively prevented.

The virus of rabies. The rabic virus is a typical example of a *neurotropic* virus—one that propagates in the susceptible host almost entirely in the nervous tissue. It is easily filtrable through all grades of filters. It is pathogenic for many animal species, and fatal rabies is readily induced experimentally by the intracerebral inoculation of guinea pigs, rats, mice, and rabbits. The virus as it occurs in the saliva of the mad dog, i.e., in a natural infection, is called *street virus*, but when this virus has been fully adapted to the rabbit by passage through a series of these animals until its virulence can no longer be increased, and it kills rabbits always in the same number of days, it has been converted into the so-called *fixed virus.* All the several forms of vaccines for prevention of rabies are prepared from a fixed virus.

Prevention of rabies; general measures, treatment of the wound. Measures which will effectively control rabies among the animal population are well known, but they are not always successfully applied, because they require public support and cooperation, particularly among the owners of dogs. In places where rabies has been common, marked success has followed vigorous efforts (1) to eliminate as far as possible all stray dogs, and (2) to enforce an ordinance requiring all other dogs to be licensed by their owners, the license to be issued only after the dog has been vaccinated against rabies. The *vaccination of the dogs* is accomplished by a single injection of rabic vaccine; it must be repeated yearly.

When a person is bitten or scratched by a dog, possibly rabid, immediate action is called for in two directions. The wound should be thoroughly cleaned at once, and a physician called who will cauterize it with *fuming nitric acid.* This cauterization is imperative; it should always be done even though immunizing injections are to be taken later.

Efforts must then be made to determine whether the dog was really rabid. It is best to capture it, confine it securely, and observe its behavior for a few days. But if, in the interest of safety, it is necessary to kill the animal, the head should be packed in ice and sent to the nearest laboratory where the brain may be examined for Negri bodies. The immunizing injections of rabic vaccine are ordinarily delayed a few days until it is known that the dog is rabid, but it may be considered advisable to begin them at once, for they may later be stopped, and no harm done, if the animal does not prove to be rabid.

Prophylactic vaccination against rabies. *The Pasteur treatment.* The method of immunization against rabies first worked out by Pasteur (1880–85) is still widely used.

As a preliminary to the actual preparation of a vaccine Pasteur experimented with the effect of the rabic virus on laboratory animals, and learned to convert the "street virus" to a "fixed virus" which would kill rabbits regularly on the seventh day.

The next step was the development of a satisfactory method of attenuating "fixed virus," so that it could be safely used as a vaccine. Pasteur found that, when spinal cords of rabbits containing the virus were dried in the air over potassium hydroxide, the virulence of the virus was gradually lost. By inoculating dogs with emulsions of cords dried for varying periods, beginning with the least virulent and working up to the fully virulent cord, he succeeded in immunizing the animals against rabies. The first trial of this preventive vaccination in a human case was made in 1885 upon a nine year old boy, Joseph Meister, who had been severely bitten by a mad dog and without the treatment would surely have died of hydrophobia. But Pasteur's method of immunization was completely successful; the boy remained well, and this bold experiment served as the starting point for a system of preventive treatment for hydrophobia which has since been used the world over.

In the scheme of treatment now most widely employed, a series of subcutaneous injections of rabies vaccine is given on twenty-one consecutive days. Each inoculation consists of an emulsion in salt solution of a small piece of a rabbit spinal cord (from a rabbit killed by "fixed virus") which has been dried for a specified number of days. The first dose of the vaccine is prepared from a cord dried for eight days; subsequent doses are made from cords dried seven days, then six, five, four, and three days. This rabies vaccine is readily secured from State health laboratories or special "Pasteur Institutes" almost everywhere.

Newer forms of rabic vaccines. A number of methods simpler than those used by Pasteur for the attenuation of fixed rabies virus have been tried, among them simple dilution (Högyes method), freezing the brain and cord with CO_2 snow and then drying the ground up material for two days over H_2SO_4 (Harris method), dialyzing a saline emulsion of the brain with formaldehyde solution (Cumming method), and finally, inactivating the virus with phenol (Semple method). Of all forms of rabies vaccines, those prepared by the last-named method are probably the most extensively used at the present time.

Complications; value of antirabic vaccination. Ordinarily the course of prophylactic inoculations against rabies is accompanied by no general reactions or marked discomfort of any kind. Adults commonly show during the second week of injections troublesome inflammations about the site of the inoculations due to the development of a local hypersensitivity, but these disappear quickly when the treatments are completed. The value of this prophylactic immunization against hydrophobia is well established. Only a small fraction of 1 per cent of persons who take the treatment ever develop rabies.

POLIOMYELITIS (INFANTILE PARALYSIS)

Nature; clinical features; manner of spread. Poliomyelitis is an acute, communicable disease, caused by a specific neurotropic *filtrable virus*, which may take a number of clinical forms, but is characterized principally by paralysis of various groups of muscles. It attacks chiefly young children—hence the common name, infantile paralysis—but it also occurs in older persons. The disease is seldom fatal, but in a high proportion of patients a partial paralysis persists for long periods, sometimes permanently, and the unfortunate individuals may be seriously crippled for life.

Poliomyelitis epidemics occur, usually in the late summer, but the distribution of cases among any group of people is very irregular. The typical disease appears in comparatively few of the persons who are certainly exposed to the infection. There is seldom more than one case in a family. However, in epidemic times many individuals show vague symptoms of variable nature, without paralysis, which are almost certainly due to infection with the poliomyelitis virus in a mild or atypical form. The virus is spread to others by these persons, and also by perfectly healthy carriers, as well as by those with the typical disease. There has been some controversy as to the way in which the infection may be spread, but most authorities agree, in view of the fact that the virus has been demonstrated in the nasal secretions of patients and carriers, that the disease is transmitted, like other infections of the respiratory tract, through personal contact and chiefly by droplet infection. There is much evidence that the virus enters the body by way of the olfactory cells of the nose, and passes up the olfactory nerves directly to the brain, and thence to the medulla and upper spinal cord where the principal lesions are located. It may be that the virus may also get into the body through the digestive tract and that the infection is transmitted by other means than personal contact, but these questions at the present time remain unsettled.

The virus of poliomyelitis. The causative agent of poliomyelitis is present in filtrates of the brain and spinal cord tissue, and may be found in filtered washings from the nasopharynx of human patients. With these filtrates the disease may be reproduced in monkeys by various routes of inoculation, including introduction of the material intranasally. The virus has not been successfully adapted to mice or other laboratory animals. It has been grown in tissue cultures. Its size has been estimated to be only about 10–20 mμ. No inclusion bodies have been identified.

Prevention of poliomyelitis. An attack of infantile paralysis leaves at least a temporary immunity, and the serum from recovered individuals possesses the power to neutralize poliomyelitis virus, as may be shown by monkey protection experiments. It has been found, too, that a large proportion of normal adults in cities have neutralizing antibodies in their blood for this virus, though they may never have suffered from clinical poliomyelitis. Naturally, convalescent serum, (or the serum from normal adults) has been tried for the prophylaxis, and also for the treatment of poliomyelitis in its very early stages. The value of such serum for these purposes was at one time regarded as considerable, but at present its practical usefulness is believed to be very slight.

Safe and efficient methods for active immunization have not as yet been worked out. The most promising prophylactic measure yet developed is the use of a *nasal spray* composed of an alum-picric acid mixture, zinc sulphate, or other astringent solution. Such a solution is capable of protecting monkeys from an intranasal instillation of the virus. Proper application of the spray to young children so as to cover effectively the olfactory area is not easy, however, and the practical value of the method still remains to be established.

ENCEPHALITIS

Types of encephalitis in human beings. In 1913 von Economo first called attention to a peculiar disease of the central nervous system characterized by the development in most patients of a marked and often long-continued state of somnolence. Following the great epidemic of influenza in 1918, sporadic cases and small outbreaks of this disease, which became known as "sleeping sickness" or *lethargic encephalitis*, were observed rather frequently in most countries, including the United States. Since about 1925, however, the affection has become increasingly rare everywhere. The cause of this form of encephalitis, now called *Type A encephalitis*, has never been definitely determined, but all available evidence indicates that the disease is an infection caused by a filtrable virus.

A second form of encephalitis, known as the Japanese, or *Type B encephalitis*, has occurred in Japan in recent years. This disease is definitely an epidemic infection, occurring almost entirely in the summer months, and is clinically more acute than Type A.

At present, in the United States, two other types of encephalitis are of particular interest: (1) the so-called St. Louis encephalitis, and (2) the similar disease that arises from infection with the virus of horse encephalomyelitis.

St. Louis encephalitis was first recognized as an epidemic disease when more than one thousand cases occurred in St. Louis in the late summer of 1933. In this epidemic, after an incubation period estimated to vary from about nine to fourteen days, patients developed an acute illness, characterized by severe headache, stiff neck and high fever, and usually fell into a somnolent state. Recovery within about ten days, without signs of residual nervous impairment, was the rule. Few children were attacked, and both the incidence and mortality increased sharply with the age of the patients. The total mortality was about 20 per cent. The disease was apparently spread by personal contact, for careful studies revealed no evidence for its transmission by mosquitoes, or the public water or milk supply. As in poliomyelitis, however, the distribution of cases was most irregular, suggesting that a peculiar susceptibility of the individual or other secondary factor accounted for the appearance of the clinically typical cases. It may be that all the sources and modes of infection are not yet understood.

The St. Louis epidemic was thoroughly investigated and one important result was the isolation of a specific *filtrable virus* which reproduced the disease in monkeys. The virus has since been studied extensively. Inclusion bodies have not been definitely associated with it. The size of the virus particles has been estimated to be 22–33 mμ. Although St. Louis encephalitis is closely similar in seasonal occurrence and clinical features to the Japanese type, experiments have indicated that the causative agent of the two forms of encephalitis are not identical. The St. Louis virus seems, indeed, to be a new virus, for it is neutralized in mouse protection tests only by the serum of convalescents and others in the St. Louis area who have been in contact with that virus, and not by the serum of persons who have recovered from lethargic encephalitis, Japanese encephalitis, poliomyelitis, or other diseases.

Another outbreak of the disease occurred in the St. Louis area in 1937, and doubtless the infection is now endemic in many parts of the country.

Equine encephalomyelitis infection. The discovery was made in 1938 that occasional cases of acute encephalitis in human beings are due to a virus which is apparently identical with that responsible for an epidemic infection among horses, known as equine encephalomyelitis. There is a suspicion that the virus may be carried from infected animals to man by mosquitoes.

YELLOW FEVER

Historical importance; early work on the cause and prevention of yellow fever. Yellow fever is an acute infectious disease, transmitted by the bites of infected mosquitoes, and caused by a specific filtrable virus. Of all the serious epidemic diseases which have affected peoples of the Western Hemisphere it has perhaps the greatest historical importance. The story of the conquest of this disease makes one of the most dramatic chapters in the history of medicine. It was yellow fever (together with malaria) that made almost uninhabitable for the white man (before about 1904) large areas of Central and South America, and the West Indies. For a time these demoralizing infections defeated attempts to build the Panama Canal. Prior to 1906 severe epidemics of this fearsome disease repeatedly appeared along the southern seaboard of the United States, and with especially devastating effect in New Orleans, where in 1878 there were, among a population of 210,000 persons, 27,000 cases and 4,046 deaths. Patients were suddenly stricken with pain in the head and back, congested face and high fever, and soon developed jaundice, severe gastrointestinal symptoms, often with black vomit, and suppression of urine. The mortality was frequently 40 per cent or more.

The first definite progress toward an understanding of yellow fever was made by the American Army Commission, composed of Reed, Carrol, Lazear, and Agramonte, working in Havana, Cuba, in 1900. With the aid of human volunteers from the ranks of the Army, this commission established the following fundamental points:

(1) Yellow fever is caused by an invisible, filtrable virus, present in the blood of patients during the first few days of the disease,

(2) The disease is transmitted from man to man solely by the bite of infected mosquitoes (*Aedes egypti,* at that time called *Stegomyia calopus*), and not by even the most intimate contact with patients or with the bedding or clothing soiled by the excreta of patients.

The investigators proved that to become infective the mosquito must bite the patient within the first three days of the illness, and that about twelve days must pass before this mosquito becomes capable of transmitting the disease to another human being. The idea that yellow fever is carried by *Aedes* mosquitoes had been strongly championed by Finlay since 1881.

The practical preventive measures suggested by these findings were soon tried out with remarkable success. In Havana and Panama, Army sanitarians, under Gorgas, undertook a vigorous campaign to destroy mosquitoes and to protect the populace from their bites, with the result that yellow fever was eradicated from these areas. Similar success followed antimosquito work elsewhere, and it was generally thought that all the necessary knowledge was at hand to eliminate yellow fever permanently from the face of the earth.

Modern studies; the menace of jungle yellow fever. As late as 1927, however, little was known about the causative virus. In that year a commission (Stokes, Bauer, and Hudson), sent to study the disease in West Africa by the Rockefeller Foundation, succeeded for the first time in producing yellow fever infection experimentally in animals, when they found the Asiatic monkey susceptible. By animal experimentation they confirmed the conclusions of the Reed Commission, and in particular reestablished the fact that the causative agent is a specific filtrable virus. A further practical advance was made when Theiler in 1931 adapted the yellow fever virus to mice by intracerebral inoculations, thus making possible the laboratory study of the virus by the same methods so successful with other viruses. Since an attack of yellow fever confers a lasting immunity, investigators were not surprised to find that the serum of persons who had recovered from the disease, even though the illness was suffered years before, possessed the power to neutralize the virus specifically in mouse protection tests. Such tests are now being extensively used to determine past incidence and present prevalence of yellow fever infection.

These laboratory tests and recent field observations have brought out several important new facts. The disease has been found to exist over much wider areas in both South America and Africa than was formerly supposed. Most important is the discovery by Soper (1935), and other investigators, that there exists an endemic focus of yellow fever in the vast jungles of South America. This *jungle yellow fever* occurs naturally in monkeys, and possibly other jungle animals, and from them is transmitted to man, as is proven by the occurrence of the disease among natives living near the jungle.

The jungle disease occurs without the presence of *Aedes egypti* and is presumably transmitted by other varieties of mosquitoes, several of which have been shown to be potential carriers, and possibly by other biting insects. It appears from this that yellow fever, far from being conquered entirely, is still a menace, for a permanent reservoir of the infection hides in the jungle.

The possibility that yellow fever may be reintroduced into communities in the United States or elsewhere where *Aedes* mosquitoes abound is a real one, and is a matter of concern for international health authorities. The increase of air travel all over the world makes it imperative that precautions be observed to prevent the unwitting transfer of infected mosquitoes on airplanes.

The virus of yellow fever. The causative agent of yellow fever is one of the smallest of the viruses, the particles having an estimated diameter of no more than 17–28 mμ. It is easily filtrable through the finest of filters. In the liver cells from monkeys and human beings dying of the disease intranuclear inclusion bodies have been found. The virus is highly infective, and is said to be able to penetrate the unbroken skin and conjunctiva of monkeys. At least five American investigators, including Noguchi and Stokes, have died while studying the disease.

Active immunization. Sawyer and associates (1934) devised a method of active immunization against yellow fever which is apparently of real value for the protection of laboratory workers and others exposed to the infection. The vaccine is composed of a mixture of mouse brain containing the virus and human immune serum.

DENGUE

This disease has many similarities to yellow fever. It is a virus infection, transmitted by the same mosquito (*Aedes egypti*) that carries the yellow fever virus. It is of more immediate interest, however, for extensive epidemics of dengue have recently occurred in the southern United States, as well as in warmer countries over seas. The onset of the illness is very abrupt. There is an original fever period of about three days, then a remission, followed by another febrile period. There is a characteristic skin rash. The severe pains in the back and joints that accompany the infection have given the disease the popular name of "break-bone fever." Little is known about the causative virus except that it is filtrable, and apparently distinct from that of yellow fever.

REVIEW QUESTIONS—CHAPTER XXXIX

1. Discuss the prevalence of smallpox in historical times and at present. What are the principal clinical features of the disease? What is variola, alastrim, vaccinia? What is the relation of smallpox to cowpox? How does smallpox naturally spread?
2. How is variola virus converted to vaccinia virus?
3. Name and describe the inclusion bodies and elementary bodies of vaccinia.
4. What method of preventing severe smallpox was practised before the days of vaccination?
5. Who developed the method of vaccination, and when? Distinguish between inoculation smallpox and vaccinia. Explain how the principle of vaccination was discovered and proven to be sound. What is the origin of the word *vaccine?*
6. Outline the modern method of preparation of smallpox vaccine from calves. What are two other sources of vaccinia virus for possible use in vaccines?
7. What principles are followed in modern methods of vaccination? Describe the multiple pressure method.
8. Name and describe the types of local reaction that may follow smallpox vaccination and explain the significance of each.
9. What are the important points to be remembered in the proper care of the vaccination wound?
10. Defend the necessity and value of vaccination for the prevention of smallpox.
11. What is the cause of rabies? Describe rabies in dogs. What are Negri bodies? How can a diagnosis of rabies be made after the death of an animal?
12. Describe rabies (hydrophobia) in man. What are some of the factors which influence the chances of acquiring rabic infection from a dog bite? What feature of the infection makes possible time for active immunization?
13. Describe the properties of the virus of rabies and the characteristic inclusion bodies.
14. What are the principal general measures that have been found effective in controlling rabies among the dog population?
15. What local treatment must be given the wounds made by the bites or scratches of a possibly rabid animal? What should be done with the animal?
16. Who developed the original method of vaccination for the prevention of rabies in human beings, and when?
17. Outline the important steps in the preparation of Pasteur's vaccine and the scheme of anti-rabic "Pasteur treatment" usually followed.
18. Describe the newer forms of rabies vaccines.
19. What complications of rabic vaccination may be observed? Is the immunization of proven value?

20. Describe the clinical features, mode of spread, and prevention of poliomyelitis.
21. What is known about the virus of poliomyelitis?
22. Name and discuss briefly the four principal types of encephalitis in human beings. Describe the virus of St. Louis encephalitis.
23. Discuss the historical importance of yellow fever and outline the early work on the cause and prevention of this disease. Name the principal insect vector.
24. Outline the nature of modern investigations of yellow fever, and the new facts which have been discovered.
25. Describe the virus of yellow fever. Is artificial active immunization practicable?
26. Describe the nature, prevalence, causative virus, and manner of spread of dengue.

CHAPTER XL

ACTINOMYCOSIS AND FUNGOUS INFECTIONS

IN CHAPTER VI we have described the general properties of *Actinomyces* and related higher bacteria. We noted there that, although there are several different types of disease with which pathogenic *Actinomyces* may be associated, the most common and most important actinomycotic infection is that typified by the "lumpy jaw" of cattle and caused by the anaerobic species *Actinomyces bovis*—the infection generally referred to when we say *actinomycosis.* This disease as it occurs in man is the subject of a brief account below.

In the same chapter the true fungi (*Eumycetes*) were described, and on pages 73–74 was presented a working classification of the principal pathogenic fungi, and of the diseases caused by them. We will not repeat that outline here, but will proceed to a short discussion of some of the more important of the fungous infections.

ACTINOMYCOSIS

Clinical features. In human beings, actinomycosis may be localized (1) in the *skin,* where nodules which form in the subcutaneous tissue eventually break down and discharge pus, (2) in the *lungs,* where the infection is often so severe that the patient dies, or (3) in the *intestine,* especially about the caecum and appendix. Most common, and characteristic of actinomycosis, however, is (4) an infection involving the tissues of the neck at the angle of the *lower jaw.*

In the typical actinomycosis of the neck and face the infected area shows a hard, brawny swelling. (In cattle, nodular swellings in the jaw account for the popular name for the disease—"lumpy jaw.") The swollen areas later become soft and discharge pus.

Usually the disease progresses very slowly, but there is a severe injury to the affected parts. The bones as well as the soft tissues are destroyed. The infection spreads through the tissues by continuity; unlike tuberculosis and syphilis, the distant lymph vessels and glands draining the area are not involved. Sometimes, if the mouth parts only are infected, the disease is slowly overcome, but often death is caused by other germs which have gotten into the injured tissues.

Actinomycosis of the lungs may resemble tuberculosis, the abdominal infection may suggest cancer or other disease, and in general the clinical manifestations are so varied that an exact diagnosis can be made only on the basis of laboratory examinations which demonstrate the presence of the causative *Actinomyces.*

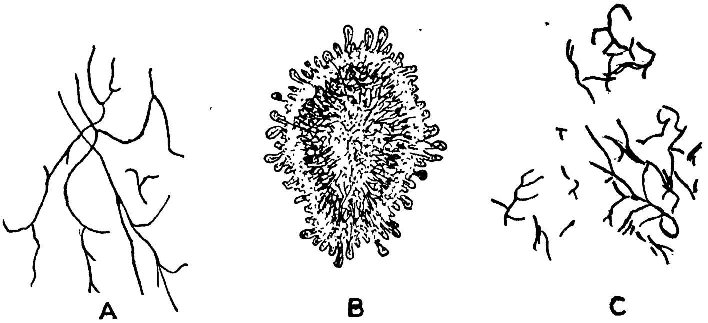

Fig. 96. Actinomyces. A, appearance of organisms in smear from a granule. B, an actinomyces granule as it appears when crushed upon a slide, showing the characteristic radiating structure with "clubs" at the periphery. C, actinomyces in smear from a culture.

Sources and modes of infection. Just how actinomycosis is acquired is not known. The infection is not passed from man to man, and many cases occur without any known contact with animals. It seems likely that the causative organism may exist naturally in the mouth of animals or man, and that infection occurs when there is a loss of the usual level of local or general resistance.

Actinomyces bovis. The causative organisms of actinomycosis appear in the pus from the lesions as yellowish, granular bodies ("sulphur granules"). These are about 2mm. in diameter and may be recognized with the naked eye. When a granule is crushed under a cover-slip on a glass slide and examined without staining, it appears as a rosette-like mass, made up of a dense network of filaments which radiate outward from the center, hence the name "ray-fungus" (Fig. 96). The curious structure of the granule is best seen if a little 20 per cent sodium hydroxide solution is added to the preparation. The free ends of the filaments at the outer edge of the granule are thickened, forming what are called actinomyces "clubs." These thickened, clubbed ends probably represent the reaction of the organism to the body fluids which surrounded the granule. If we apply the Gram stain, the central mass of filaments stains Gram-positive, but the clubs appear Gram-negative.

Laboratory diagnosis. A diagnosis of actinomycosis is made when the characteristic sulphur-yellow granules, made up of the ray-fungus as above described, are found in the pus. The yellow granules must be searched for, fished out with a loop or capillary pipette, and crushed under a cover-slip.

If cultures are attempted they should be made with material from a previously unopened abscess. Good results have been reported by making the primary inoculation of the pus into deep columns of dextrose agar by the stab method. Once in pure culture, the organisms grow fairly readily, and show the characteristic coherent, dry, or chalky colonies previously described.

SPOROTRICHOSIS

Clinical features. This is a specific infection of the skin and underlying tissues, usually of the arm, characterized by gumma-like swellings and ulcerations along the course of lymph vessels, and caused by the fungus *Sporotrichum schenki.* The disease begins with a small sore on the back of the hand, or on other exposed portions of the body. This initial lesion can often be traced to the prick of a thorn, and undoubtedly is the point of inoculation of the fungus. The infection usually runs a protracted, mild course, extending slowly along the lymph channels. At points along the vessels, swellings develop which are at first hard, then later soften and discharge pus. Sometimes the fungus becomes more widely disseminated in the body and may involve

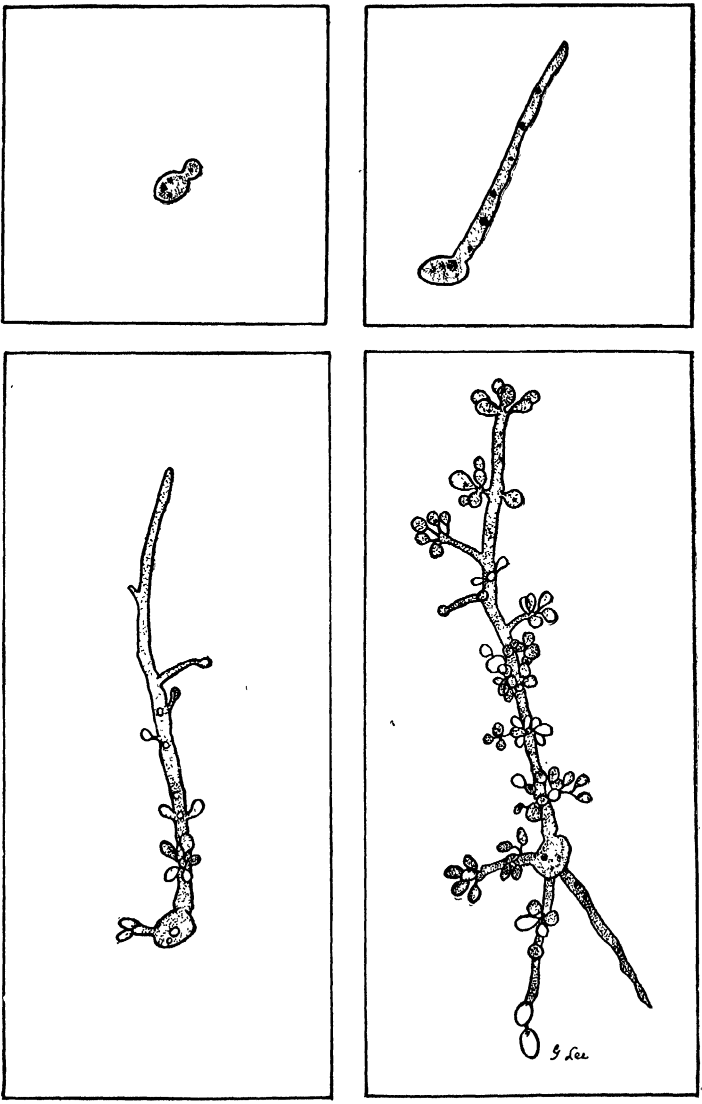

FIG. 97. *Sporotrichum schenki.* Stages in the growth from a chlamydospore. (Original preparations by Dr. John R. Schenken.)

various tissues, such as muscles, bones and joints, and the lungs. Clinically the disease may be confused with syphilis or tuberculosis.

Sources and modes of infection. The manner in which infection with *Sporotrichum* is acquired is not definitely known. It apparently occurs when plant material bearing the causative organism is mechanically carried into the skin as by a thorn prick or other superficial wound.

Sporotrichum schenki. The causative fungus of sporotrichosis was first described by Schenk in 1898. In the pus of the lesions the organisms are ordinarily too few in number to be seen. They grow out readily, however, in cultures on Sabouraud's medium. Colonies appear in three or four days, and microscopic examination reveals a delicate, septate, branching mycelium, approximately 2 μ in diameter, to which are attached numerous oval or pear-shaped reproductive spores. These occur singly along the mycelium or in cloverleaf-like clusters at the extremity of short branches (Fig. 97).

Laboratory diagnosis depends on culturing the organisms. This is best done by obtaining pus from a previously unopened lesion.

BLASTOMYCOSIS

Clinical features. Blastomycosis is a specific infection caused by a particular fungus, generally known as *Blastomyces hominis* or *Blastomyces dermatitidis*. In its most common form blastomycosis is chronic, and involves the *skin* only. Beginning as a small papule on the hand, face, neck, or other exposed part, it spreads slowly and irregularly into the surrounding skin. In these cases there is usually little systemic illness, and after several years, recovery may ensue. In other cases, however, the internal organs are invaded by the organisms, and abscesses may form in various locations. Usually the first lesions are in the *lungs*. Later the kidneys, bones, central nervous system, and other regions are involved, and the patient succumbs to an overwhelming infection. The clinical course may suggest tuberculosis, syphilis, or sporotrichosis, and exact diagnosis depends upon laboratory examinations.

Blastomyces hominis. The causative organism of blastomycosis is a typical example of a fungus with properties intermediate between those of the common filamentous molds on the one hand, and those of the simple, budding yeasts on the other. In the body tissues the fungi appear as yeast-like budding cells, but in laboratory cultures they develop a filamentous growth—a moderately luxuriant mycelium (Fig. 98). In the pus from the lesions (or in sections of the tissue) the organisms are seen as round or oval bodies, 7 to 20µ in diameter, with thick, doubly-contoured walls. They are often surrounded by a capsule. Many of the cells show *buds* forming from them.

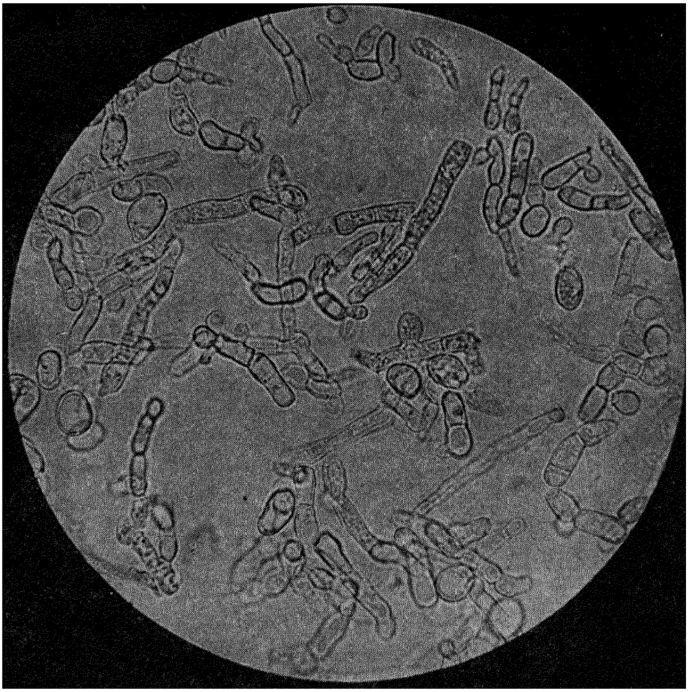

FIG. 98. *Blastomyces hominis* in pure culture.

The *Blastomyces* may be cultured readily on glucose agar, or other media, at room or incubator temperature. In culture the organisms show considerable variations in the manner of growth with the passage of time, and on different types of media. The colonies are originally smooth and creamy, somewhat similar to those of staphylococcus, but eventually become covered with a spiny or downy overgrowth which represents an aerial mycelium. The morphology of the organisms is irregular, and is correlated with the colony form. At first they appear as they do in tissue as yeast-like bodies reproducing by budding, but soon there appear many elongated cells which seem to be preliminary to the development of a filamentous growth. Still later a coarse, irregular, branched mycelium appears.

Laboratory diagnosis. Microscopic examination of the pus from the lesions (or in pulmonary cases of the blood-stained sputum) will show the characteristic encapsulated, budding forms. For complete diagnosis the fungus should be isolated in pure culture and studied for several weeks to establish its properties.

COCCIDIOIDAL GRANULOMA

Clinical features. This is a specific infection caused by *Coccidioides immitis.* This disease is more dangerous than most other fungous infections, for it often causes death within a few months or years after its onset. Recent studies indicate, however, that mild cases ending in full recovery are relatively more frequent than fatal ones. These mild cases may show skin lesions (erythema nodosum), and commonly the lungs are infected, causing clinical signs and symptoms which often suggest tuberculosis. In the fatal cases the skin, or the lungs, may be the site of the initial infection, but the organisms are soon carried by way of the blood stream to internal organs and the infection rapidly becomes generalized.

Sources and modes of infection. The majority of reported cases of coccicioidal granuloma in the United States have originated in the San Joaquin valley in California. A few cases have been noted in other states, and the disease has also been recognized in Mexico and Brazil. The manner of infection is not definitely known, but it seems likely that the dried spores of the fungus are inhaled, or are introduced from dirt or plant material through skin abrasions. An especially large proportion of cases occur among men who are workers in the fields, or vineyards.

Coccidioides immitis. This fungus, first described by Wernicke (1892) was originally regarded as a protozoan of the genus *Coccidium,* hence the name. It was later shown to be a fungus which, like the *Blastomyces,* appears in the tissues as a round body without filaments, but develops a mycelium in culture. In the thick pus from the lesions the organisms are easily recognized as cyst-like, spherical bodies, varying in size from about 5μ to as much as 60μ or more in diameter, and having a thick, refractive, doubly-contoured capsule. Within the rounded elements may be seen replicas of the parent organism, consisting of tiny, round structures enveloped in a doubly-contoured capsule. These represent endospores and indicate the method by which the organism reproduces itself in the tissues. These bodies somewhat resemble the *Blastomyces,* but are distinguished by the *presence of endospores* and by the fact that *there are no buds.*

These fungi may be isolated readily on a variety of culture media. They grow best at 37° C. and on blood agar, in brain broth, or in similar enriched media. The cyst-like structures described above are never seen in culture; instead the organism develops at once a filamentous growth like an ordinary mold. The colonies which appear in a few days on solid media are at first creamy white plaques, but they soon become fuzzy with the development of an abundant aerial mycelium. Microscopically, the growth appears as a tangled network of long, profusely branched, septate filaments, about 3μ wide, with many chlamydospores.

Laboratory diagnosis. The characteristic thick-walled cyst-like structures, with endospores and without buds, are so characteristic and numerous in the pus that their recognition is easy. Cultures will reveal the organism in its filamentous phase.

MONILIASES

Clinical types of moniliasis. There is a large group of fungous infections of the mucous membranes, skin, and other tissues, which are attributed to yeast-like organisms classified in the genus *Monilia.* The best known of these infections is called *thrush.* This is an acute inflammation of the mucous membrane of the mouth, gums, tongue, or pharynx, characterized by the appearance of a whitish, membranous patch over the inflamed area, resembling the membrane of diphtheria. In shreds of this membranous material the causative organisms are easily found. Thrush is quite common in ill-nourished and ill-kept children. The infection may be carried readily from child to child in a hospital ward unless care is exercised.

Besides the typical thrush, a similar infection with *Monilia* may be seen in the angle of the mouth, about the genital and anal region, or between the fingers and toes, in infants and young children. In adults, *Monilia* have been found associated with a great variety of infections involving the *skin,* the mucous membranes of the *mouth* and *vagina,* and in inflammations of the *bronchi and lungs.* In tropical countries, and also occasionally in the United States, appear cases of *sprue.* Patients usually have a sore throat, diarrhea, and a severe anemia. In the intestinal discharges yeast-like organisms of the *Monilia* type are often conspicuous, and for a long time these organisms were regarded by many authorities as the true cause of the disease. It is now believed, however, that sprue is primarily a deficiency disease related to pernicious anemia.

The pathogenic Monilia. The name most commonly applied to the organism found in thrush and in similar infections of the mucous membrane and skin, is *Monilia albicans.* The variety associated with sprue has long been known as *Monilia psilosis.*

Monilia albicans appears in scrapings from the lesions of thrush or from infected skin areas, principally as oval, budding cells 5 to 7μ in diameter. Also there may be seen a few slender, branched, septate threads, surrounded by clusters of the budding cells. The organism may be cultured readily upon any sugar media. The colonies on solid media are creamy, moist and smooth, much like those of ordinary yeasts. In these surface colonies the organisms have the morphology of budding yeasts. In the depths of liquid media, however, or below the surface in agar or gelatine stab cultures, and especially when growing in corn meal agar, there occurs an outgrowth of mycelial threads. The filaments are made up of elongated cells which are not readily broken apart. At the end of these threads and along their course develop dense ball-like clusters of blastospores, formed by budding. Chlamydospores are also present.

Laboratory diagnosis. In view of the fact that yeast-like fungi similar to the pathogenic *Monilia* are widely distributed, a diagnosis of moniliasis must always be made with caution. However, the typical *Monilia albicans* is rarely present on the healthy skin, and if the characteristic budding organisms are found constantly in abundance in a particular lesion they may be safely assumed to be the causative fungi. Cultures should be studied and animal inoculation should be performed. The full identification of a strain of *Monilia* requires the patient effort of a well trained mycologist.

TORULOSIS

This is a relatively rare disease caused by infection with the pathogenic yeast *Torula histolytica* (*Cryptococcus hominis*). The torulae are not found in skin inflammations. Their portal of entry into the body is probably the lungs, and a few cases have definite lung involvement, but they cause the greatest damage when they reach the central nervous system, where they produce a *fatal infection of the brain and meninges.* The symptoms may suggest brain tumor, tuberculous meningitis, or encephalitis.

Torula histolytica, like ordinary yeasts, reproduces only by budding, and never forms mycelium, but is distinguished from the *Saccharomyces* by the fact that it fails to form ascospores or any other type of reproductive spore. In the infected meninges little masses form containing groups of the organisms, each enclosed in a gelatinous capsule. These masses may be large enough to be seen with the naked eye. They resemble the tubercles of tuberculosis, but are larger and more opaque.

Laboratory diagnosis may sometimes be made by finding the yeast-like cells, which have a diameter of 3–4μ, in the spinal fluid, but more commonly the organisms are first demonstrated in smears from the brain or in sections of the tissue obtained at autopsy. Cultures on glucose agar or other solid media yield white or yellowish, smooth, glistening, heaped-up colonies, similar to those formed by the saprophytic yeasts. Unlike the latter, however, the *Torulae* are virtually inert in culture; they do not ferment carbohydrates.

RINGWORM OR TINEA

Most common of the fungous diseases of man are those infections of the skin, hair, or nails, generally referred to as *ringworm* or tinea. These infections may be caused by any one of many closely related, varieties of *fungi imperfecti.* Large areas of the skin may be involved, but the mucous membranes, and the inner tissues are not invaded. On the skin the lesions frequently take a circular form, thus making a ring of infected tissue. Many of the ringworm infections, especially those common in children, are contagious, and must be guarded against in schools, children's hospitals, and similar institutions. Ringworm is a common disease in dogs, cats, horses, and other domestic animals, and many cases in human beings are acquired by contact with infected animals.

The ringworm fungi. The micro-organisms responsible for ringworm infection, often spoken of as the *dermatophytes,* have no special characteristics which serve to differentiate them from other fungi imperfecti, nor are there many properties which may be used to divide the numerous varieties into definite genera or species. They do not form distinctive structures for the bearing of reproductive spores, but small, pear-shaped conidia develop in most cases along the sides or in clusters at the ends of undifferentiated hyphae. In addition to these small spores, many of the dermatophytes form those curious, elongated, spindle-shaped, multichambered bodies called fuseaux (Fig. 14). These fungi all grow slowly, forming eventually, dense colonies which usually become covered with a downy or powdery growth of aerial hyphae. Because of their complexity and variability their systematic classification on purely mycological grounds is difficult and unsatisfactory. It is customary to follow the suggestions of Sabouraud, who met this difficulty by arranging these fungi into three main genera according to their appearance in or about infected hairs:

(1) *Microsporum*—forms which develop a mass of small, round spores forming a collar about the base of the hair just beneath the skin surface;

(2) *Trichophyton*—all other varieties that invade the hair; and

(3) *Epidermophyton*—fungi that do not attack the hair, but invade the epidermis only.

Diagnosis of ringworm is made by carefully removing infected hairs from the area involved (usually hairs that come out most easily are the diseased ones), mounting them in sodium hydroxide solution under a cover glass, and noting the size, position, and arrangement of the spores.

REVIEW QUESTIONS—CHAPTER XL

1. Describe the clinical and pathological features of actinomycosis. What is the origin of human cases?
2. Name and describe the causative organism as it appears in pus from the lesions.
3. What practical methods are used in the laboratory diagnosis of actinomycosis?
4. Outline the clinical features of sporotrichosis and discuss the possible sources of infection.
5. Name and describe the causative organism and state how laboratory diagnosis is made.
6. Describe the clinical features of blastomycosis. What can be said about the sources of infection?
7. Name and describe the causative fungus as it appears in the infected tissues and in cultures.
8. What are the practical methods used in laboratory diagnosis of blastomycosis?
9. Describe the clinical features of coccidioidal granuloma and explain its prevalence and possible sources of the infection.
10. Name and describe the causative fungus as it appears in the infected tissues and in cultures.
11. What procedures are used in making a laboratory diagnosis of coccidioidal granuloma?
12. Describe the various forms of infection attributed to *Monilia*.
13. Discuss briefly the general properties of the pathogenic *Monilia*. Name two species commonly recognized. Describe the properties of *Monilia albicans*.
14. How is a laboratory diagnosis of moniliasis made?
15. Name and describe the causative organism of torulosis. What is the final outcome of torula infection? How is the laboratory diagnosis made?
16. What is meant by "ringworm"? Describe the general properties of the ringworm fungi. Name and define the three genera of the common ringworm fungi generally recognized.

CHAPTER XLI

AMEBIASIS. MALARIA

IN CHAPTER V the general properties of the *Protozoa* were described, and the principal species of medical interest were mentioned. Trypanosomiasis and some of the other diseases caused by protozoa were also discussed. We have reserved for this chapter, however, an account of the two protozoan diseases of most outstanding importance in the United States—namely, amebiasis and malaria.

AMEBIASIS

Clinical and pathological features. Infection with the pathogenic ameba *Endameba histolytica* is called *amebiasis.* This organism invades the wall of the large intestine, producing an ulcerative colitis, which, if sufficiently severe, causes diarrhea and the symptoms of *amebic dysentery.* Not infrequently the ameba are carried by the blood stream through the portal veins to the liver and there they set up an amebic *liver abscess.* Rarely, other regions of the body are the site of secondary localizations of the organisms.

The onset of amebic dysentery is usually less abrupt, and the symptoms less severe than in bacillary dysentery. The incubation period is commonly about three weeks. The disease runs a protracted course, periods of freedom from general illness alternating with periods of more or less acute gastrointestinal distress. There is much evidence that cases with mild symptoms only, or with no noticeable symptoms at all, far outnumber the cases with frank, clinical dysentery. Different individuals show a marked difference in resistance to this infection; whereas some are so susceptible that the amebae proliferate vigorously, produce large ulcerations and scars of the intestinal walls, and otherwise cause severe damage, other persons suffer only minimal lesions. Once infected, and in the absence of adequate treatment, an individual is likely to remain a carrier of the amebae indefinitely.

Prevalence; sources and modes of infection. Amebiasis is prevalent in most of the warmer countries of the world. Like typhoid fever and bacillary dysentery it tends to be more common wherever sanitation is not the best. In the United States clinical cases of amebic dysentery are not numerous, but amebic infection of the mild or symptomless type is probably more prevalent than is generally realized.

The only way one can acquire infection with *E. histolytica* is to swallow the *cysts* of the ameba. The real source of amebic infection, then, is the excreta from convalescent patients and symptomless carriers, for these are the individuals who are excreting cysts. The cysts are carried about in the same ways, and by the same means, that operate to spread the germs of typhoid fever, cholera, and other bacterial diseases of the intestine. Cooks and other food handlers contaminate food or water with the fingers. Flies also may convey the cysts to food. A public water supply may be contaminated. In general, the cysts of this dangerous ameba are more resistant than the pathogenic intestinal bacteria; they are not killed, for example, by the concentration of chlorine ordinarily used in drinking water.

The dire possibilities of water-borne infection was illustrated in a dramatic fashion by the extraordinary outbreak of more than 800 cases of amebiasis originating in Chicago in 1933. This outbreak occurred among the employees and guests of two of the large hotels. Investigations disclosed that a cross-connection existed between the drinking water intake pipe and a sewer pipe. Cysts resembling those of *Endameba histolytica* were demonstrated in the water pipe near this cross-connection.

Endameba histolytica. In the mucus of the diarrheal stools from the acute case of amebic dysentery, in material obtained with the aid of the proctoscope from the base of ulcers in the rectum, or in preparations from the walls of amebic abscesses, the active, ameboid, growing forms of the organism,—the *trophozoites,*—may be found. These are about 20–30μ in diameter. They reproduce in the tissues by fission. When observed unstained they show a grayish, finely granular, translucent endoplasm, and a definitely deliminated, clear, highly refractile ectoplasm. There is a single, delicate nucleus, hard to make out unless especially stained. The organisms are actively phagocytic and their cytoplasm contains *red blood cells* and sometimes other tissue elements, but, unlike the saprophytic amebae, no bacteria (Fig. 99).

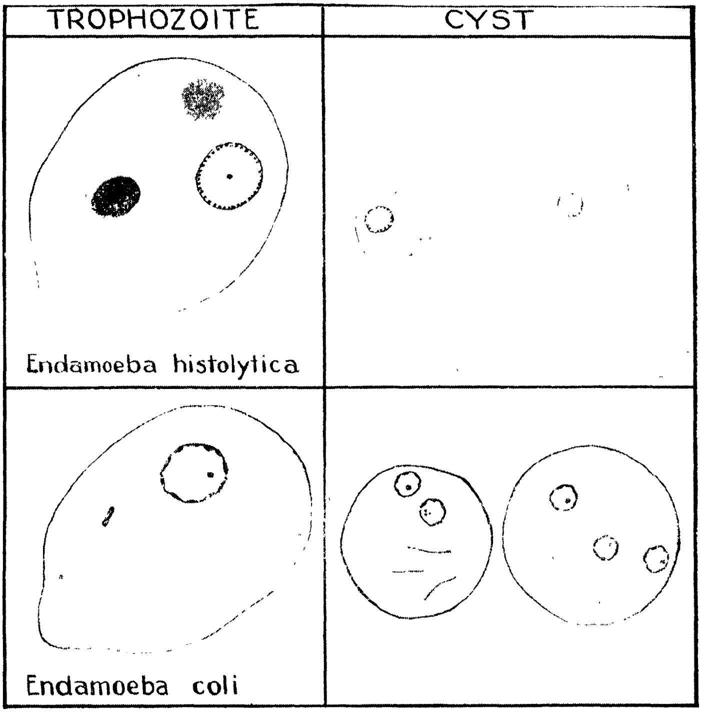

FIG. 99. Illustrating the principal differences between the trophozoites and cysts of *Endameba histolytica* and of *Endameba coli.* (Reproduced through the courtesy of Dr. Emma S. Moss.)

Unless expelled at once, amebae that leave the infected tissues and are caught in the intestinal contents undergo encystment. *Cysts* are found in the feces in subacute or symptomless stages of amebic dysentery. The process of encystment proceeds in several stages, finally resulting in a heavy-walled round or oval body 10 to 15μ in diameter, containing *four* small nuclei (Fig. 99).

Laboratory diagnosis. The definite diagnosis of amebiasis demands that the presence of either the trophozoites or the cysts of *Endameba histolytica* be demonstrated in the intestinal discharges of the patient. A distinction must be made between this pathogenic ameba and *Endameba coli*, the harmless ameba normally present in the feces. This is not always easy, and considerable experience and judgment are required. The principal points of differentiation are illustrated in Figure 99.

MALARIA

Prevalence and importance. Malaria is one of the most common and widespread of all human ills. It occurs throughout the world in the temperate and torrid zones, and is especially frequent and severe in tropical countries. Malaria has played an important part in the history of nations and it is still so common that it constitutes one of the most serious drawbacks to the development of peoples in many parts of the world. Before the introduction of malaria control measures, which were first applied successfully on a large scale about 1900, the danger to life and health from this one disease was so great in certain regions, as for example in the Roman Campagna (the great plains about the city of Rome, Italy) and in parts of India and South and Central America, that large areas of otherwise desirable land were rendered uninhabitable.

The Isthmus of Panama was for centuries one of the most unhealthy spots for the white man in all the tropical world because of malaria and yellow fever (both mosquito-borne diseases). As we have previously explained, it was American army sanitarians who succeeded in conquering yellow fever and in greatly reducing the amount of malaria in Havana and in Panama by fighting the mosquitoes, and their work made possible the building of the Panama Canal, begun in 1904. This highly successful work stimulated similar efforts to control malaria and other mosquito-borne diseases in other parts of the world.

Malaria is still excessively prevalent, however, in many places. In the United States, within a single year, literally millions of persons are affected. The proportion of cases which result in death is very small, but in many southern communities a large fraction of the population is chronically ill and as a result the whole region suffers a tremendous economic loss. In the past few years, however, there has been a gradual reduction in the prevalence of malaria in the South as control measures have been instituted more widely.

Discovery of the malaria parasites and of the rôle of mosquitoes in the transmission of malaria. "Chills and fever" was a familiar disease even to ancient peoples. The curious course of the illness, during which the patient has a chill, then fever, then a period without symptoms, followed by chills and fever again, was long ago recognized as characteristic of some peculiar disease. It was also known long before the true explanation of the fact was found that malaria is most frequent in the vicinity of swamps and marshy land. It was believed that poisonous vapors from such places polluted the air and this poisoned air was supposed to cause the disease, hence the name *mal-aria*, which means literally "bad air."

The first step toward an understanding of malaria was made in 1880 when Laveran, a French military surgeon, described the protozoa of malaria in the blood. Later (1885–90), Golgi and other Italian investigators worked out the cycle of development of the malaria parasites in the human red blood cells. The rôle of mosquitoes in transmitting the disease was first clearly shown (1896–98) by Ross, a British army surgeon working in India, who proved that bird malaria is mosquito-borne, and also saw the parasites of human malaria in *Anopheles* mosquitoes and predicted that these insects would be shown to be the transmitters of the human disease. His pioneer studies were soon confirmed by other workers. The complete life cycles of the three chief types of parasites causing human malaria were worked out, and it was established that it is through the bite of infected *Anopheles* mosquitoes and only in this way that malaria parasites are carried from one person to another.

The malaria parasites and their life history. The germs of human malaria are protozoa belonging to the class *Sporozoa*, to the order *Hemosporidia*, and the genus *Plasmodium*. They are exclusively parasitic, and lack any special organs for locomotion or for the ingestion of food. They form spores at one stage of their life cycle. They live in the blood stream and parasitize the human red blood cells.

Species of malaria parasites. There are three principal species of these parasites that produce malaria in human beings. *Plasmodium vivax* causes the form of malaria called *tertian* (or *benign tertian*), in which chills and fever occur every third day, that is, at about forty-eight hour intervals. *Plasmodium malariae* causes the so-called *quartan* type of malaria, in which chills and fever occur every fourth day, at seventy-two hour intervals. *Plasmodium falciparum* is the cause of the severe and often fatal form, common in the tropics, called *estivo-autumnal* or *malignant tertian* malaria. In this type the interval between attacks of fever is irregular, but often averages about forty-eight hours. Persons are often bitten more than once and on successive days by infected mosquitoes, so that there may be two or more sets of parasites maturing at different times with the result that attacks of chills and fever may occur daily or at irregular intervals. The life cycle of all three of these species is essentially the same. There are two distinct phases: (1) in the red blood cells of the human body, and (2) in the mosquito (Fig. 100).

Asexual cycle in human red blood cells. The parasites are present in the secretions of the salivary glands of infected *Anopheles* mosquitoes. They pass with this secretion into the proboscis of the mosquito at the time of biting and thus are injected into the human blood stream. The parasites penetrate into red blood cells; only one plasmodium enters a single cell in most cases. The parasite begins to grow within the blood cell, assuming at first a "signet-ring" appearance; then later it becomes a large irregular-shaped body nearly filling the entire cell. This process of growth requires from about forty-eight to seventy-two hours, depending on the species of *Plasmodium.* Finally the parasite becomes divided by asexual division (*schizogony*) into a number of tiny spore-like bodies—the *merozoites.* These are young forms, and like the one which originally entered the cell, are capable of penetrating other red blood cells. The blood cell by this time is entirely destroyed, and it ruptures, setting free the merozoites in the blood stream. Many of these die at once or are destroyed by phagocytes, but some succeed in invading other red blood cells and so complete the cycle. At the time the infected red cells break up, releasing the young parasites, it is supposed that poisons are liberated and these poisons are thought to cause the characteristic chill.

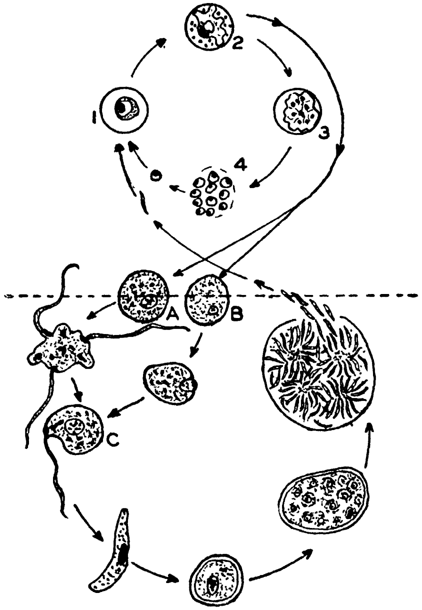

Fig. 100. Diagram illustrating the life cycle of the tertian malarial parasite (*Plasmodium vivax*). The stages *above* the dotted line occur in the blood of man, whereas those *below* the line are found only in the mosquito. 1, 2, 3, 4, stages of the asexual cycle in human red blood cells. 4, the merozoites, produced by asexual multiple division (schizogony). A, the microgamete (male cell), and B, the macrogamete (female cell), sexual forms of the parasite. After fertilization of the female cell within the mosquito (C), a series of changes occur which eventually result in the production of very many tiny spindle-shaped forms, the sporozoites, by the process called sporogony. These forms reach the salivary glands of the mosquito, and, when this infected mosquito bites again, these sporozoites are inoculated into the blood and invade red blood cells, thus completing the cycle from man to mosquito and back again. (Modified from Hegner, Cort, and Root.)

This *asexual cycle* (Fig. 100: 1, 2, 3, 4) goes on continuously from the time the first parasites are introduced by the mosquito bite, more and more blood cells being infected, until, after ten days or two weeks (the usual incubation period of the clinical disease) several hundred cells in every cubic millimeter of blood are infected. The classical symptoms then appear. The parasites almost always set up a regular rhythm of development (less definite in *P. falciparum* infections), so that in all the blood cells they mature at the same time. The sudden bursting of so many erythrocytes and the consequent release of so much poison causes the appearance of the chill, and from then on the chills and fever recur at regular intervals, according to the species of the parasite concerned.

After the asexual development of the organisms as described above has gone on for a number of days, new forms begin to appear within a small proportion of the infected red blood cells. These new forms are *gametocytes*, the *sexual forms* of the parasites. These develop rather slowly, some become male cells (*microgametocytes*), others female cells (*macrogametocytes*). They do not multiply or otherwise change, once fully matured in the human red blood cell, but they undergo a developmental cycle of their own within the body of *Anopheles* mosquitoes (Fig. 100: A, B). The person with gametocytes in the peripheral blood, where these mosquitoes may get them, is the "carrier" of malaria.

Sexual cycle in the mosquito. When the sexual forms are taken into the stomach of the mosquito, as she bites a malaria patient, the male cell throws off tiny bodies resembling human spermatozoa, which have an active movement. One of these bodies enters and fertilizes a female cell which in the meantime has matured to receive it. The fertilized cell now becomes embedded in the stomach wall of the mosquito and proceeds to grow into a large round cyst-like body, the *oocyst.* A single mosquito may have scores of these oocysts in the stomach wall. In eight or ten days the oocysts become filled with hundreds of delicate sickle-shaped forms—the *sporozoites.* (These are the "spores" which give the name *Sporozoa* to this class of protozoa. Their formation is referred to as *sporogony* in contrast to schizogony, the asexual process by which merozoites are formed). The sporozoites soon escape into the body cavity of the mosquito and become distributed throughout the body of the insect, many of them lodging in the salivary glands. Then, when the mosquito bites, these sporozoites are introduced into the human blood stream and start once more the asexual cycle in the human red blood cells.

It takes about ten to fifteen days for the development of the parasites within the mosquito, and until these changes are complete the insect is not able to infect human beings. Once the parasites have reached the salivary glands, however, a mosquito may infect many persons in succession. The mosquito does not seem to be harmed in any way by the presence of the malaria parasites.

Laboratory diagnosis of malaria. A positive diagnosis is made by finding the parasites in the blood of the patient. A drop of blood from the ear or finger may be examined at once under a microscope and the ameboid movement of the living parasites within the red blood cells may be seen. An easier method is to make the usual type of thin blood smear upon a glass slide and stain it by Wright's or Giemsa's stain. Blood for examination is best secured either just before or a few hours after the chill. The parasites will be larger before the chill, but are likely to be more numerous after the chill.

Treatment of malaria. Malaria is one of the few diseases for which we have specific chemical remedies, the principal ones being *quinine* and related derivatives of chinchona bark. Quinine has been known and used since the seventeenth century. Two synthetic products have recently been added to the list of drugs useful in malaria therapy—*plasmochin*, which destroys the sexual forms (gametocytes) in human blood but has practically no effect on the asexual forms, and *atabrine*, which, like quinine, attacks the asexual parasites actually causing the illness in man. Combinations of plasmochin and quinine are sometimes used in an attempt to free the blood entirely of both the sexual and asexual parasites.

These drugs have remarkable therapeutic value, and when properly applied will bring about a complete cure in the great majority of cases. It is necessary however that sufficiently large doses be taken over a sufficient length of time. When quinine is used, in order to be certain that all parasites have been killed, it is necessary to continue taking the drug for eight weeks or more after the symptoms have disappeared. Often this is not done, and in districts where malaria is common, there are many persons who never receive adequate treatment. Such individuals harbor the parasites in small numbers and have a mild, chronic form of malaria, or have periods of freedom from all symptoms which are interrupted by relapses every now and then. Some of these persons have gametocytes in the circulating blood, and constitute the source from which *Anopheles* mosquitoes are infected, with the result that malaria is kept alive in the community.

Prevention of malaria. A marked reduction in the amount of malaria can be brought about in almost any territory if modern knowledge is intelligently applied. The principal measures used in successful malaria control work include: (1) adequate treatment of all cases, (2) protection of the people against mosquito bites by screening of dwellings, and (3) destruction of mosquitoes by elimination or treatment of their breeding places,

REVIEW QUESTIONS—CHAPTER XLI

1. Define *amebiasis*, and name the causative organism. What are the chief clinical and pathological features?
2. Discuss the prevalence of amebiasis. How is infection acquired and how are the amebae passed from person to person?
3. What is meant by *trophozoite, cyst?* Describe the trophozoites and the cysts of *E. histolytica.* May the organism be cultivated?
4. Discuss the historical importance of malaria and its prevalence at the present time.
5. What is the literal meaning of the word *malaria?* What is the origin of the term?
6. Who discovered the malaria parasites and when? Who first described the development of the parasites in mosquitoes?
7. Outline the general properties of the malaria protozoa. Name the three species which infect human beings and state the type of disease which each causes.
8. Trace the life cycle of malaria parasites in human red blood cells. At what stage of development does the chill occur? What is the incubation period in malaria and what happens during this period? Describe the sexual forms and explain their importance. Define *schizogony, merozoite, gametocyte, microgametocyte, macrogametocyte.*
9. Trace the development of the sexual forms of the malaria parasites within the mosquito. How long after a mosquito has taken in the parasites before the mosquito can infect another person? Define *oocyst, sporogony, sporozoites.*

10. Describe the methods used for demonstrating the presence of malaria parasites in the blood. When is blood best taken for microscopic examination?
11. What well-known drug has a specific curative effect on malaria? Name two synthetic products for malaria therapy, and explain their action on the parasites. Discuss the value of adequate treatment as a malaria-preventive measure.
12. Name three measures which are helpful in the prevention of malaria.

APPENDICES

APPENDIX A

COMPARISON OF METRIC AND ENGLISH UNITS OF MEASURE AND CENTIGRADE AND FAHRENHEIT TEMPERATURE SCALES

IN ORDINARY life in English-speaking countries we measure length by inches and feet, but in scientific work linear measure is based upon the *metric system,* and length is expressed in terms of *meters* and multiples and fractions of a meter. The standard meter has a length of 39.37 inches. In microscopical sciences, such as bacteriology, the metric units in most common use are *centimeters* (a hundredth part of a meter), and *millimeters* (a thousandth part of a meter). A centimeter is about $\frac{3}{8}$ of an inch, and a millimeter about $\frac{1}{25}$ of an inch. A useful mental picture of these units of measure, as compared with a familiar scale in inches, may be gained by study of the accompanying diagram (Fig. 101). In the metric scale here shown the smallest divisions are in millimeters, and the numbered divisions in centimeters.

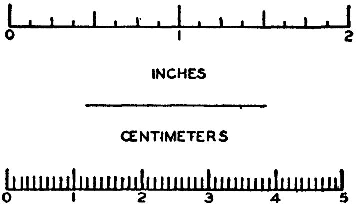

Fig. 101. Scales for comparison of English and metric units of linear measure. The smallest divisions of the lower scale are millimeters.

Bacteriologists have found it necessary to adopt a unit of measure still smaller than the millimeter in order to express the extremely minute size of bacteria and other micro-organisms. This is the *micron* (μ), or micromillimeter, which is a *thousandth part* *of a millimeter.* Most of the bacilli are no more than one micron (1µ) wide, which means that a thousand of them could be laid side by side across a millimeter space.

In the metric system the unit of *weight* is a *gram.* This is the weight of one *cubic centimeter* of water. The unit of *volume* is a *liter,* which is *1,000 cubic centimeters,* or a little more than a quart.

In all scientific work, and, in fact, even for ordinary purposes practically everywhere except in English-speaking countries, temperature is expressed according to the *Centigrade* scale rather than the more familiar *Fahrenheit* scale. In the latter the freezing point of water is 32° and the boiling point 212°, while according to the *Centigrade* scale water freezes at 0° and boils at 100°. Thus, the range of temperature from the freezing to the boiling point on the *Fahrenheit* scale is divided into one hundred and eighty degrees and the same range of temperature on the *Centigrade* scale is divided into only one hundred degrees, and each degree *Fahrenheit* is 5/9 of a degree *Centigrade.* To change a *Fahrenheit* temperature reading to its equivalent on the *Centigrade* scale subtract 32, then divide by 9, and multiply the result by 5. To change a *Centigrade* reading to its equivalent in the *Fahrenheit* scale divide by 5, then multiply by 9, and add 32. The accompanying figure will help to familiarize the student with the relation between these two scales (Fig. 102).

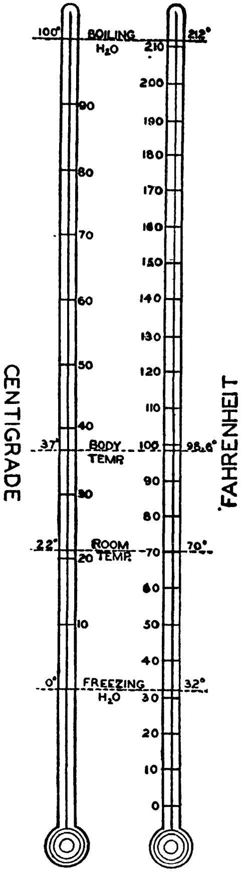

FIG. 102. Laboratory thermometers, for comparison of the *Fahrenheit* and *Centigrade* scales.

Clinical thermometers are made with either the *Fahrenheit* or the *Centigrade* scales, and some physicians prefer one, and some the other. The normal body temperature is 98.6° F., or 37° C., and a temperature of 104° F. is equivalent to 40° C. The diagram (Fig. 103) permits a ready comparison of the two scales. It is so drawn that the equivalent temperatures are directly opposite each other throughout the scale.

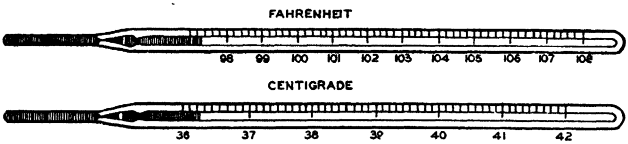

FIG. 103. *Fahrenheit* and *Centigrade* clinical thermometers. The equivalent temperatures are directly opposite each other throughout the scale.

APPENDIX B

FORMULAE AND METHODS FOR THE PREPARATION OF COMMON CULTURE MEDIA, REAGENTS, AND STAINS

PREPARATION OF STAINS

IN BACTERIOLOGY the stains known as anilin dyes are employed. The most useful of these dyes, listed according to the color they impart to bacteria, are as follows:

Red: basic fuchsin, safranin.
Blue: methylene blue, toluidine blue.
Violet: gentian violet, crystal violet.
Brown: Bismarck brown.

The most commonly used of these stains are methylene blue, gentian violet, and fuchsin. The general method of preparing staining solutions of these dyes is to make first a *saturated alcoholic solution*, then dilute this saturated solution with water or some other reagent according to some accepted formula. Directions for making the saturated solutions usually accompany the package of the dry stain when purchased. The following are the approximate amounts required to make a saturated alcoholic solution:

Methylene blue......	1.5	grams per 100 c.c. 95% alcohol
Crystal violet........	14.0	grams per 100 c.c. 95% alcohol
Methyl violet........	15.5	grams per 100 c.c. 95% alcohol
Basic fuchsin........	8.5	grams per 100 c.c. 95% alcohol

A completely saturated solution may be obtained by adding an excess of the powdered dye to the alcohol and allowing the solution to stand for several days, shaking the mixture repeatedly. If an undissolved residue remains the solution is saturated. It must always be filtered before use. The saturated solutions should be stored in the dark in glass-stoppered bottles.

For some purposes aqueous solutions are made directly from the dry stain. In the following paragraphs are given methods for the preparation of the solutions for actual use in the staining of bacteria.

Methylene blue. The most widely used preparation of methylene blue is the so-called *Loeffler's alkaline methylene blue.*

Mix the following:

Dilute KOH (1/10,000 solution in water)	100 c.c.
Saturated alcoholic solution methylene blue	30 c.c.

Shake and filter.

To make the potassium hydroxide solution dissolve 1.0 gm. of potassium hydroxide in 100 c.c. of distilled water, then take 1.0 c.c. of this solution and add to 99 c.c. of distilled water. This makes a 1–10,000 KOH solution.

Toluidine blue.

Weigh out

Toluidine blue dry powder	0.25 Gm.

Dissolve in mixture of

Absolute alcohol	5 c.c.
2.0 c.c. glacial acetic acid in distilled water	100 c.c.

Basic fuchsin. This dye is usually made up into the stain called *carbol fuchsin.*

Measure and mix

Saturated alcoholic solution basic fuchsin	10 c.c.
5% solution carbolic acid in water	100 c.c.

Filter.

Gentian violet. Various formulae have been devised for making up stains from this dye and the related crystal violet and methyl-violet dyes. The older formulae produced staining solutions which were so unstable that the stain became useless in a short time. Among the many formulae suggested to overcome this difficulty, those of *carbol gentian violet, Hucker's ammonium oxalate crystal violet, Kopeloff and Berman's alkaline gentian violet,* and *Stirling's gentian violet* have been recommended.

Carbol gentian violet.

Measure and mix

Saturated alcoholic solution gentian violet	10 c.c.
5% solution carbolic acid in water	100 c.c.

Filter.

Hucker's ammonium oxalate crystal violet.

Solution A

Crystal violet	4 Gms.
95% alcohol	20 c.c.

Solution B

Ammonium oxalate	0.8 Gm.
Water	80.0 c.c.

Mix Solutions A and B. Filter.

Kopeloff and Berman's alkaline gentian violet.

Solution A

Gentian violet (or crystal violet)	1.0 Gm.
Water	100 c.c.

Solution B

Sodium bicarbonate	1.0 Gm.
Water	20 c.c.

Just before use, mix 30 drops of Solution A with 8 drops of Solution B.

Safranin. This stain is most useful as a counter-stain in the Gram staining method.

Safranin, dry powder	2.0 Gms.
Distilled water	100 c.c.

Dissolve and filter.

Bismarck brown. This is also used principally as a counter-stain in the Gram method.

Bismarck brown	0.5 Gm.
Distilled water	100 c.c.

Dissolve by boiling, cool and filter.

SPECIAL STAINING PROCEDURES

Gram staining method. The procedure for carrying out the Gram stain has already been given in the text, p. 174. The following modifications of the Gram staining technique have been recommended recently.

Hucker's modification. In this method the ammonium oxalate gentian violet solution given above is used. Lugol's iodine solution is employed:

Iodine	1.0 Gm.
Potassium iodide	2.0 Gms.
Water	300 c.c.

Dissolve the solids in 25 c.c. of the water by heating.
Add the remaining water, shake, and filter.

The counter-staining solution is prepared as follows:

Safranin (2.5% solution in 95% alcohol)	10 c.c.
Water	100 c.c.

Method. Cover smear for 1 minute with gentian violet solution; wash in water, and then cover smear with iodine solution for 1 minute; wash in water and dry by pressing between filter papers. Decolorize in 95% alcohol for 30 seconds; cover with counter-stain for 10 seconds; wash in water and dry smear.

Kopeloff and Berman's modification. In this method the alkaline gentian violet solution given above is used. The iodine solution is prepared as follows:

Iodine	2 Gms.
Normal solution sodium hydroxide	10 c.c.

Dissolve and make up to 100 c.c. with water.

The counter-stain is made up as follows:

Basic fuchsin	0.1 Gm.
Water	100 c.c.

Method. Stain 5 minutes or more with the alkaline gentian violet solution; rinse with iodine solution; add more iodine solution and allow to stand 2 minutes or longer; drain off iodine solution and blot dry (without washing); decolorize with acetone; dry in air, counter-stain for 10 to 30 seconds; wash in water, and dry smear.

Staining of spores.

Abbott's spore stain. Cover fixed smear with Loeffler's methylene blue and heat to boiling over the flame. Repeat the heating at intervals during one minute; wash in water. Decolorize in the following solution:

80% alcohol	98 c.c.
Nitric acid	2 c.c.

Wash in water; then stain with the following solution:

Saturated alcoholic solution of eosin	10 c.c.
Water	90 c.c.

Wash in water and dry smear.

Staining of capsules.

Hiss capsule stain. The smear should be made from a serum or ascitic fluid medium or from a body fluid, such as the peritoneal fluid, and preferably without use of water. Allow the smear to dry without heating and do not fix. Cover smear with an aqueous gentian violet solution (5 c.c. saturated alcoholic solution gentian violet in 95 c.c. water), and heat gently until steam is just visible. Wash off stain at once with 20 per cent solution of copper sulphate (use no water); blot dry. The capsule appears as a faint purplish halo about the bacteria.

Gin's method of demonstrating capsules. Dilute a small amount of India ink with sterile water. Place a drop of this ink near the end of a very clean slide and carefully mix in a loopful of the bacterial suspension. Spread the mixture across the slide with the edge of a second slide (see Fig. 41) so as to produce a thin, even smear, gray in color. Allow smear to dry in air, fix, and stain by methylene blue or other simple stain. The margin of the capsule is sharply delineated by the ink and the margin of the bacterial cells by the stain.

Staining of metachromatic granules.

Albert's stain (Laybourn's modification). Stain for five minutes with the following solution:

Toluidine blue	0.15 Gm.
Malachite green	0.2 Gm.
Glacial acetic acid	1 c.c.
95% alcohol	2 c.c.
Distilled water	100 c.c.

Wash in water. Apply Lugol's iodine solution for 1 minute. Wash and dry smear.

Neisser's stain. Apply the following stain for 30 to 60 seconds:

Methylene blue	0.1 Gm.
95% alcohol	2 c.c.

Glacial acetic acid	5 c.c.
Distilled water	95 c.c.

Wash in water, and apply the following solution for 30 seconds:

Bismarck brown	0.2 Gm.
Water	100 c.c.

Wash in water and dry smear.

Staining of flagella. Of the several methods for staining flagella the method of Plimmer and Paine (a modification of the Cesares-Gil flagella stain) is one of the most successful. There must first be prepared a mordant solution as follows: (A mordant is a compound applied before staining to bind the stain).

Mordant solution

Tannic acid	10 Gms.
Aluminum chloride	18 Gms.
Zinc chloride	10 Gms.
Basic fuchsin	1.5 Gms.
60% alcohol	40 c.c.

Place the solids in a mortar, add 10 c.c. of the alcohol and mix very thoroughly, taking care to break up all the zinc chloride. Add the rest of the alcohol slowly. This alcoholic solution will keep for years at low temperatures. It must be diluted just before use according to the directions below.

Method

1. Clean slides very thoroughly. Wash them in soap and water, rinse, cover with a light paste of Bon Ami, allow to dry. Rub off the Bon Ami with a clean towel, and carefully remove all dust. Do not touch the surface of the slide at any time with the fingers. Use forceps to hold the slide.
2. With a well cleaned loop place one or two drops from the culture to be examined in a small amount of distilled water warmed to 37° C. Incubate at 37° C. for thirty minutes. (The culture must consist of active young organisms.)
3. Warm the cleaned slides to body temperature, and with the thoroughly cleaned, well flamed loop, transfer *one drop* from the surface of the distilled water culture on to the end of the glass slide. Tilt the slide so that the drop quickly flows down the slide, forming a thin film. The slide should be warm enough so that the drop dries almost at once. *Do not heat the slide.*
4. Dilute *one part* of the stock solution of the mordant with *two parts* of distilled water and filter. Allow the filtrate to drop

upon the dry film on the slide, and let it act for 60 seconds in the cold.

5. Very gently rinse off the mordant with distilled water, then apply an ordinary staining solution of carbol fuchsin for five minutes. Wash in water, and dry smear without blotting.

Staining of spirochetes. Many of the spirochetes found in the normal mouth and on the genitals or elsewhere may be stained by the ordinary gentian violet and carbol fuchsin staining solutions. Special procedures are necessary, however, for the *Treponema pallidum* and other pathogenic species. *Giemsa's stain* (described below as a blood stain) will stain spirochetes, including *Treponema pallidum,* fairly well, but it must be allowed to act for several hours. Methods commonly used for the demonstration of pathogenic spirochetes in dried smears are the *India ink method* and *Benian's relief stain* described on p. 171, the *nigrosine relief stain,* and *Fontana's staining method.*

Fontana's staining method. Three solutions are used as follows:

Solution I

Formalin	2 c.c.
Acetic acid	1 c.c.
Water	100 c.c.

Solution II

Tannic acid	5 Gms.
Water	83 c.c.
Phenol (as a preservative)	1 c.c.

Solution III

5 per cent aqueous solution of silver nitrate. Add drop by drop with a capillary pipette ammonium hydroxide until a precipitate forms and redissolves. To this solution add more silver nitrate until a solution is produced which remains slightly cloudy on shaking.

Method. Prepare smear in the ordinary way but do not heat the slide. Cover smear with Solution I for about one minute, then wash in water. This fixes the smear. Flood smear with Solution II and warm slide gently until the solution steams, and let it act for 30 seconds longer, then wash in water. This is the mordant solution. Now cover smear with Solution III, warm slide until the solution steams, and allow it to act for 30 seconds. Wash in water, and dry smear. Spirochetes appear dark brown or black.

Nigrosine stain. The staining solution is prepared as follows:

Nigrosine (Coleman and Bell)	5 Gms.
Distilled water	100 c.c.

Boil 30 minutes in an Erlenmeyer flask; then add as preservative:

Formalin	0.5 c.c.

Filter twice through double filter paper and store.

Method. To a loopful of exudate on a clean slide add a loopful of the nigrosine solution and spread the mixture carefully by rotary movements so as to obtain in some portion of the smear the optimum thinness. Dry rapidly over the flame, apply oil and examine directly with the oil immersion objective. The *Treponema* stand out clearly against the dark background as in a dark-field microscope. For preservation of the slide remove oil by immersing in xylol solution for a time. Drops of exudate may be allowed to air *dry* on a slide and some days later may be wetted by applying a drop of water, then stained by following the usual procedure as outlined above.

Preparation and staining of blood smears. It is often necessary to examine a drop of blood with the microscope. Malaria and various other diseases of the blood are diagnosed by examination of stained blood smears. Such preparations are also used in making a "differential count" of the white blood cells, that is, a count to determine the proportionate number of the different types of leukocytes present. To make the smear a *small* drop of blood is placed near the end of a *perfectly clean slide.* This drop is then smeared out to form a thin film, using the edge of another slide, in the manner described above for India ink smears (Fig. 41).

The most commonly used stains for blood smears are *Wright's stain* and *Giemsa's stain.* These are called *polychrome* (many-color) stains, because they impart different colors to different structures. The nucleus, cytoplasm, and granules of the various types of white blood cells, take characteristic colors and by their staining the cells may be identified. It is best to purchase these stains already prepared from the manufacturer.

Wright's stain. This stain is applied in the following manner: Cover the dry blood smear with the stain by means of a medicine-dropper, and *count the drops.* After one minute add the same number of drops of distilled water. Allow the mixture to remain on the smear for 3 to 6 minutes. Then flood the slide quickly with water and wash in water for about 30 seconds, or until the thinner portions of the blood film become yellow or pink in color. Dry smear by warming slide very slightly.

Giemsa's stain. This stain is applied in the following way: Fix the blood smear by covering it for 3 to 5 minutes with methyl alcohol. Wash off the alcohol with water. Then immerse the slide in a jar containing a freshly prepared mixture of 1 c.c. of Giemsa staining solution and 10 or 15 c.c. of distilled water. The action of this mixture is intensified by the addition of a drop of a 1 per cent sodium carbonate solution. 10 or 15 minutes is sufficient to stain most bacteria and protozoa, but for some spirochetes several hours may be necessary. After the staining, remove slide, wash in running tap water, and dry.

PREPARATION OF NUTRIENT BROTH AND AGAR

Nutrient extract broth (1 liter).

1. Measure 1,000 c.c. of tap water into a pan. Mark the level of the water on the side of the pan or on a stick (*e.g.*, a tongue depressor) held vertically in the center of the pan.

2. Weigh out the following:

Beef extract	3 Gms.
Peptone	10 Gms.
Salt (NaCl)	5 Gms.

3. Heat the water to boiling. Add the beef extract, and the salt, then dust the peptone on to the surface a little at a time and stir. Boil until all ingredients are thoroughly dissolved.

4. Add water to make up to the original volume.

5. And NaOH if necessary to adjust the reaction of the medium to about the neutral point, using one of the methods outlined below.

6. Boil medium for 10 minutes, add water to restore the original volume, check the reaction and readjust if necessary.

7. Allow broth to cool to about 50° C.

8. Filter through nonabsorbent cotton in a large funnel, and distribute to test tubes, or flasks.

9. Plug the tubes and flasks with cotton, and sterilize the medium in the autoclave for 20 minutes at 15 pounds pressure.

Infusion broth (1 liter).

1. Allow finely chopped, lean beef to stand with tap water in the ice box over night (about 1 pound of meat to a liter of water).

2. Separate the fluid from the meat, preferably by use of a

metal sieve, and press as much of the juice out of the meat as possible. Measure and make up to 1,000 c.c., with water. Add 10 grams of peptone and 5 grams of salt, and proceed as in making extract broth.

Extract or infusion agar (1 liter).

1. Cut 15 grams of shredded agar into short lengths, place in large beaker or graduate and cover with cold tap water.
2. Prepare extract (or infusion) broth as above through step 6.
3. Drain the excess water from the agar shreds, and add the agar to the broth. Heat the medium until the agar is *completely dissolved,* stirring continuously. Add water to restore the original volume.
4. Prepare a filter of absorbent cotton in a large funnel. Soak the cotton with hot water, then *press out as much of this water as possible.*
5. While the agar is still hot pass it through the cotton filter, and into tubes or flasks. For *slants,* fill tubes about ¼ full; for *deeps* fill tubes about ⅔ full. When tubing do not get the agar about the neck of the tube. Filtering and tubing must be done rapidly, for otherwise the agar will solidify.
6. Plug the tubes and flasks with cotton and sterilize in the autoclave for 20 minutes at 15 pounds pressure.
7. On removal from the autoclave and before the agar has cooled, place the tubes intended for slants in a slanting position until the medium has solidified.

Adjustment of the reaction of culture media. This very important step must be conducted with care whenever the medium is to be used for critical work. Space forbids the description here of the more accurate methods now generally used by bacteriologists for determining reaction. In order to understand these methods and the principles involved the student should consult the *Manual of Methods for Pure Culture Study of Bacteria* (Society of American Bacteriologists), Tanner's *Practical Bacteriology* (John Wiley & Sons, N. Y., 1928), *Clark's Determination of Hydrogen Ions,* 3rd Ed. (Williams & Wilkins Co., Balt., 1928), or other authorities listed in Appendix C.

For many purposes a satisfactory broth or agar medium may be prepared, and the general method of adjusting reaction of media may be experienced by the following simple procedure:

Test the reaction by removing a few drops of the medium to a porcelain test plate and adding to it a drop of a solution of the indicator called *brom thymol blue* (or *phenol red* may be used). Broth medium is always acid before adjustment; both of these indicators will give a yellow color in this case. Add to the medium normal sodium hydroxide a drop or two at a time, stir, and test the reaction repeatedly until brom thymol blue gives a *grass green* color (neither yellow green nor blue green) or until phenol red is faintly and permanently pink. At this point, which can easily be recognized, the medium is nearly *neutral.* The colors given by the indicators may be compared with those in a chart, such as may be found in Clark's book referred to above, or better, to the colors given by the same indicators in solutions of known reaction prepared according to directions given in the books referred to above.

REAGENTS AND TECHNIQUE USED IN COMMON BIOCHEMICAL TESTS

Indicators. One of the best of the indicators especially adapted for bacteriological work, is Andrade indicator.

Andrade indicator. This is prepared by dissolving 0.5 Gm. of *acid* fuchsin in 100 c.c. of distilled water, and adding N/1 NaOH to the solution until it changes to a brownish color, and then to yellow (about 17 c.c.). 1 c.c. of this indicator is added to 100 c.c. of medium. Acid formation causes the indicator to turn a pinkish red.

Tests for indol formation. *Ehrlich's test.* To 3 c.c. of the broth culture (forty-eight hours or more old) add 1 c.c. of a 2 per cent solution of para-dimethylamidobenzaldehyde in 95 per cent alcohol, and ten drops of concentrated hydrochloric acid. Indol gives a rose-red color.

Goré's modification of Ehrlich's test. The following solutions are used:

Solution I:

Paradimethylamidobenzaldehyde	1 Gm.
Ethyl alcohol (95%)	95 c.c.
Hydrochloric acid (concentrated)	20 c.c.

Solution II:

Saturated aqueous solution of potassium persulfate ($K_2S_2O_8$).

Procedure:

1. Remove the cotton plug from the culture tube.
2. Moisten the under surface with 4–6 drops of Solution II, then an equal amount of Solution I.

3. Replace the plug and push it down to within an inch of the surface of the medium.

4. Place the tube upright in a bath of boiling water and heat for 15 minutes.

5. Remove the plug and examine its under surface. A pink (rose) color on the plug indicates indol.

Nitrate reduction test (production of nitrites). The following solutions are used:

Solution I.

5N acetic acid	1,000 c.c.
Sulphanilic acid	8 Gms.

Solution II.

5N acetic acid	1,000 c.c.
Alpha-amidonaphylamine	5 Gms.

Dissolve, then filter through absorbent cotton.

Procedure. To cultures in nitrate broth or agar (i.e., media containing 0.1 per cent peptone and 0.02 per cent nitrite-free potassium nitrate), about 4 days old, add 2 c.c. each of the above reagents. A rose red color indicates the presence of *nitrites.* Check by control test on uninoculated medium.

Fermentation reactions. *Cultures in liquid media.* Note the presence or absence of growth in the closed arm, and correlate this finding with the capacity of the organism to ferment the carbohydrate present. Determine the reaction of the medium with litmus paper, or by adding phenol red, or brom thymol blue indicator. For a more accurate determination of the pH, remove 0.5 c.c. of the medium to a test plate and add an indicator, comparing the color produced with that given by standard buffer solutions of known pH.

To determine roughly what proportion of the gas in the closed arm is CO_2, mark on the arm the point to which the gas reaches, fill bulb of tube completely with a NaOH solution, close with thumb, invert and shake the tube thoroughly, then allow remaining gas to collect in closed arm again. The difference between the volume of gas now and before admixture with NaOH represents the amount of CO_2. The remaining gas is usually H.

Cultures in soft agar. Fermentation of carbohydrates in soft agar media is indicated by a change in the color of the indicator. Distinguish carefully between the color changes which are a direct result of fermentation of the added carbohydrate and those due to fermentation of the small amounts of dextrose likely to be present in the basic agar medium. Observe the tubes at frequent intervals for several days to detect changes. A control (uninoculated) tube which has been incubated with the inoculated tubes should always be available for comparison.

Note evidences of gas production along line of inoculation. Distinguish gas bubbles carefully from bubbles of air or cracks in the medium.

Production of H_2S. One of the best methods for testing the capacity of bacteria to produce H_2S is to make stab inoculations into a soft agar medium devised by Treece. Development of black color about the growth is a positive sign of production of hydrogen sulphide. Treece medium is made up as follows:

Prepare separately a 2 per cent solution of ferrous sulphate and a 2 per cent solution of sodium thiosulphate. Sterilize these solutions by autoclaving. Add 1 c.c. of each of them (with aseptic precautions) to each 90 c.c. of a melted, sterile, soft nutrient agar (pH 7.4), and tube the medium aseptically.

Bile solubility test (for differentiation between pneumococci and streptococci).

Divide the young broth culture (or the saline suspension of the young organisms) to be tested into two portions (of about 2 c.c. each) in separate small test tubes. Add about 0.5 c.c. of sterile, clear ox-bile or a 10 per cent solution of sodium taurocholate to one tube, and an equal amount of salt solution to the other (control) tube. Compare the appearance of the liquids in the two tubes at once and at intervals in the next half hour. Usually pneumococci dissolve and the originally cloudy suspension clears up immediately, even at room temperature. Streptococcus cultures remain cloudy.

APPENDIX C

SOURCES OF INFORMATION ABOUT MICROBIOLOGY

THE ORIGINAL sources of information concerning microbiology consist in the published researches of scholars in the field. These are to be found in many different journals, monographs, and other scientific medical publications. The titles of papers written upon any one subject can be assembled in a short time by consulting the *Cumulative Index Medicus* to be found in any medical library. The student should learn the use of this and other reference books in the libraries, and experience first-hand the pleasure of "looking up" the latest published work in a particular phase of microbiology. The habit of glancing through scientific journals, and noting the matters discussed, is recommended even for the beginning student, for this is perhaps the fastest way to make the strange new facts encountered in microbiology seem real, and to learn that the great practical value of this subject in connection with everyday problems, claimed for it by textbook and instructor, is no academic boast, but the simple truth. A few of the most prominent bacteriological journals in the English language are:

American Journal of Hygiene
American Journal of Public Health
American Journal of Tropical Medicine
British Journal of Experimental Pathology
Journal of Bacteriology
Journal of Experimental Medicine
Journal of Immunology
Journal of Infectious Diseases
Journal of Laboratory and Clinical Medicine
Journal of Pathology and Bacteriology (British)
Journal of Preventive Medicine
Journal of Tropical Medicine and Hygiene (London)
Public Health Reports (U. S. Public Health Service)

There are numerous excellent books on microbiology, immunology, and public health. A *partial* list of those useful for reference, arranged by topics in the general order of the subject matter of this textbook, is presented here.

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APPENDIX D

SUGGESTED LABORATORY EXERCISES AND DEMONSTRATIONS FOR THE STUDENT NURSES' COURSE IN MICROBIOLOGY

General considerations. The exercises and demonstrations outlined below must be regarded as suggestive only. It would be quite impossible to give a set of laboratory exercises which would be suitable for use in every school of nursing. There are so many different ways in which the laboratory work might be arranged effectively, that no one program could be rightly regarded as the best under all circumstances. In general, the teacher will be guided, of course, by the excellent outline for *Microbiology* in the *Curriculum Guide for Schools of Nursing*, published by the National League of Nursing Education (1937). The individual instructor will have to decide, according to the facilities, help, and time available, what is most suitable for his class. The author believes, however, that the general plan of the exercises here described may be followed with advantage, even though the actual work in the various laboratory periods may be much simplified, expanded, or changed, according to circumstances, and the wishes of the instructor.

The purpose of the laboratory work should be very clear in the mind of the instructor. In general, the primary purpose is to bring the subject of microbiology "down to earth," and to give it a practical reality for the student. It is intended to make the nurse "microbe-conscious" through actual experience with bacteria and other microbes in a laboratory, and to supply her some tangible and accurate ideas about the nature of "germs" in general. Of course, she may also gain some specific information about particular organisms, and about methods of bacteriological diagnosis, so that she can visualize more clearly the exciting cause of common diseases, and can appreciate the kind of specimens needed for successful laboratory examinations.

The laboratory work should give some training in the elements of aseptic technique. In the author's experience the best way to accomplish this, and at the same time to assure the interest of the student, is to require individual members of the class to handle independently as many cultures as possible and to carry out faithfully the necessary elementary bacteriological technique. In so doing, the student will learn the necessity for asepsis as in no other way. It should be clearly understood, however, that in requiring students to carry out technical bacteriological procedures the technique is not taught for its own sake, or with the intention of making bacteriologists of the nurses, but merely in order that the students may better enjoy the course, and derive the pleasure and profit that comes from first hand experience. Some demonstrations, in which the student takes no part, must be included in any program, but the more of the work the students do themselves the better.

Fifteen three hour class periods at weekly intervals, from one to two hours of each period being devoted to laboratory work, and the remaining time to lecture, recitation, or demonstration, is a satisfactory arrangement, although a course of sixty hours or more is much to be preferred, in view of the ever-increasing extent of the subject. Just before the students begin the laboratory work, the instructor should actually perform the various required steps in the day's exercises, explaining methods as he goes along. Students will then do their work much more rapidly.

Students should be required to keep a laboratory notebook according to a prescribed form. This notebook should contain a systematic account of all work done and all demonstrations given, and some original statements of new facts learned and conclusions drawn.

In connection with the study of pathogenic organisms, it is highly desirable, whenever possible, to supplement the regular exercises with demonstration of such specimens, cultures, or tests, from patients in the hospital as may happen to be obtainable. It is also helpful to exhibit pathological autopsy specimens, such as a tubercular lung, a heart showing vegetations of endocarditis, or similar illustrative material.

Suggested program.

PERIOD 1

Instructor:

Demonstrate construction, use, and care of the microscope.

Methods of examining living micro-organisms.

Students:

Examine scum of a hay infusion.

Examine yeast cells from a yeast cake.

Examine with the naked eye, and under a binocular (dissecting) microscope, various cultures and natural growths of molds.

Examine prepared microscopic slides showing the fruiting bodies of the common varieties of molds.

PERIOD 2

Instructor:

Review of the microscope. Demonstrate the use and principle of the oil immersion lens.

Demonstrate handling of test tube cultures, and preparation and staining of smears.

Students:

Stain, examine, and draw prepared slides containing smears of bacteria illustrating the principal morphological classes and groupings of the bacteria.

PERIOD 3

Instructor:

Review handling of test tube cultures, and preparation and staining of smears.

Demonstrate making of a hanging-drop preparation.

Students:

Prepare smears from the several cultures furnished containing bacteria of different morphological classes (such as diplococci, staphylococci, streptococci, tetracocci, sarcinae, bacilli, and spirilla). Stain the smears with methylene blue, examine, describe, and draw.

Examine, describe, and draw prepared smears showing bacteria with (*a*) spores, (*b*) capsules, (*c*) flagella.

Examine hanging-drops, prepared by the instructor, showing motile, and nonmotile bacteria.

Period 4

Instructor:

Demonstrate methods of pouring and inoculating agar plate cultures.

Demonstrate the Gram staining method.

Students:

Each *group* of students inoculate 2 agar plates (furnished, or poured by the students) as follows:

Plate 1. Remove the cover and expose to air for 15 minutes.

Plate 2. Brush hair over plate, touch lips to agar, touch fingers to agar, or inoculate in some other similar way.

Each *student* inoculate 2 agar plates as follows:

Plate 3. Use some object of everyday use. Inoculate in manner suggested by instructor.

Plate 4. Sprinkle or blow dust from table or floor upon the agar.

Make smears close together on the same slide from the cultures of a Gram-positive organism and a Gram-negative organism furnished. Stain both smears at the same time by the Gram staining method. Examine and describe the results.

Two (or more) students carry through the routine procedure for hand disinfection, and under supervision of instructor make plate cultures from hands and nail scrapings before and after scrubbing and after use of a disinfectant.

Period 5

Instructor:

Demonstrate methods of studying and describing agar plate cultures, and fishing of colonies to secure pure cultures.

Demonstrate result of cultures made at Period 4 from the hands before and after scrubbing.

Students:

Members of the various groups study the agar plate cultures made at Period 4. Write a systematic account of the amount, and general character of the growth obtained on all the plates made by the group, and describe in your own words the various types of colonies present. Make smears from colonies of different kinds, and stain them by a simple stain or by Gram's stain. Put these slides away for examination at the next period.

Fish a single isolated colony from any plate and transplant to an agar slant.

Period 6

Instructor:

Demonstrate ingredients used in the preparation of culture media, and types of media in common use.

Demonstrate cultures of bacteria showing putrefaction, fermentation, chromogenesis, anaerobic growth, and other activities.

Students:

Examine the smears made from the plate cultures at Period 5, and complete observations on these cultures.

Examine the agar slant culture made from a plate colony at Period 5 to determine whether or not a pure culture has been obtained.

(Test or review may be given.)

Period 7

Instructor:

In presence of the class prepare demonstrations to show the effect of sunlight, cold, heating at 65° C. and boiling, chemical disinfectants, and concentrated solutions (osmosis), on bacterial life. These demonstrations may be more or less elaborate as circumstances warrant.

Students:

Using sterile swabs, make smears from the mouth (the gum margins in particular), and from the nose and throat, and stain them with gentian violet. Examine, describe and draw.

Examine hanging-drops, or dark-field preparations, set up by the instructor with material from the gum margins.

Swab the gums and teeth, cheek, nose, or throat of a neighbor and streak a blood agar plate with the material thus obtained.

Period 8

Instructor:

Show the results of the demonstration cultures made at Period 7 to illustrate the effect of environmental conditions on bacteria.

Demonstrate the construction and operation of the dry heat oven and the autoclave.

Students:

Make a study of the growth obtained on the blood agar plate inoculated at Period 7, and compile a connected account of observations on the micro-organisms of the mouth and throat.

Period 9

Instructor:

Demonstrate and discuss practical aspects of immunity:

- Preparation of bacterial vaccines.
- Laboratory animals and their uses. Production of immune serums.
- Bleeding of animals.

Bleeding of human beings. Collection of serum (Instructor bleed a volunteer from the arm vein, separate the serum by centrifuging after the blood has clotted.)

Demonstrate method of preparing exotoxins in the laboratory (laboratory filters).

Period 10

Instructor:

Review and discuss practical aspects of immunity.

Demonstrate agglutination, using *Eberthella typhi* and antityphoid serum, in test tubes (macroscopic method), and in hanging-drops (microscopic method).

Demonstrate the Widal test. Use blood of students if possible.

Demonstrate blood tests for syphilis (Wassermann, Kahn, and Kline tests).

Demonstrate and explain use of Schick test materials, Dick test materials, tuberculin.

Demonstrate commercial preparations of antitoxin, other therapeutic serums, and vaccines.

Students:

Examine hanging drops showing positive Widal test, and control.

Perform Widal test upon yourself if desired.

Period 11

Instructor:

Demonstrate blood agar cultures of *Streptococcus hemolyticus* and *Streptococcus viridans.*

Students:

Make smears from cultures of *Streptococcus, Staphylococcus aureus,* and *Bacillus pyocyaneous* (*Pseudomonas aeurginosa*). Stain, examine, describe, and draw.

Examine, describe, and draw prepared smears of *Clostridium tetani* and *Clostridium welchii.*

PERIOD 12

Instructor:

Demonstrate method of making nose and throat cultures.

Students:

Make smears from cultures of *Corynebacterium diphtheriae*. Stain with methylene blue, examine, describe, and draw.

Stain prepared smears from positive throat cultures. Examine for diphtheria-like bacilli.

Make a throat culture from your neighbor, using a sterile swab and tongue depressor according to the technique demonstrated.

Examine prepared smears of the *Meningococcus*.

PERIOD 13

Instructor:

Demonstrate cultures of *Pneumococci*.

Demonstrate autopsy on white mouse, killed by intraperitoneal injection of *Pneumococci* and agglutination test and capsule swelling (Neufeld) test for determination of type.

Students:

Stain smears from a case of acute gonorrhea by Gram's method. Examine, describe, and draw typical pus cells containing *Gonococci*.

Examine smears showing *Pneumococci* with capsules prepared from mouse peritoneal fluid.

Examine prepared slides showing the *Treponema pallidum* in sections of the liver of a syphilitic fetus. Draw and describe.

PERIOD 14

Instructor:

Demonstrate methods for laboratory diagnosis of typhoid fever (blood cultures, Widal tests, feces cultures).

Students:

Stain prepared smears of *Eberthella typhi*. Examine, describe, and draw.

Examine, describe, and draw prepared smears from the feces of (*a*) infants, (*b*) adults.

PERIOD 15

Instructor:

Demonstrate acid-fast staining methods.

Demonstrate cultures of tubercle bacilli and other acid-fast bacilli.

Students:

Stain prepared smears made from the sputum of a case of pulmonary tuberculosis by the Ziehl-Neelsen acid-fast staining method. Examine, describe, and draw the tubercle bacilli.

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